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# Structure and function of palpi in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

by

©

Avalokitesvara Sen

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Doctor of Philosophy

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# ABSTRACT

The role of maxillary and labial palpi in host plant selection by adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) was investigated. The ultrastructure of sense organs on the apical tips of the maxillary and labial palpi and the galeae were examined using SEM and TEM techniques. Detailed chemical analysis was done on potato leaf surface waxes. Behavioral analyses were conducted to examine mechanisms of host plant discrimination and to identify components in surface waxes of potato which are stimulatory.

Based on SEM and TEM studies, there are 185 sensilla on the apex of each maxillary palp and 65 on that of each labial palp. Most sensilla on the maxillary palpi are olfactory while approximately equal numbers of gustatory and olfactory sensilla are present on the labial palpi. The galeae have gustatory sensilla only.

Bioassays conducted with beetles with their palpi waxed results in a significantly longer time to take the first bite on potato leaf discs. Of solanaceous species tested, potato offered least resistance to feeding in naive beetles as indicated by its lower values for time taken for first bite. Other species in order of decreasing preference were *Solanum elaeagnifolium, Datura stromonium, S.nigrum, Lycopersicon esculentum* and *Nicotiana tabacum.* The order of preference was similar when surface waxes extracted from leaves of these six species were offered on millipore filter

discs.

Detailed chemical analysis was done on leaf surface waxes of potato using FTIR, GC and MS. Of the different fractions tested, the chloroform fraction, consisting essentially of esters and of primary and secondary alcohols, appeared to contain the stimulating compounds.

It is suggested that palpation serves to increase ventilation and that olfactory sensilla on the maxillary palpi perceive volatile components of leaf surface compounds. Leaf surface compounds thus provide cues which induce feeding and serve as 'biting stimulants'. The high proportion of gustatory sensilla present on the labial palpi and galeae appears appropriate since these organs are in more frequent contact with the leaf before and during feeding.

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# INTRODUCTION

Phytophagous insects exhibit specialized feeding habits. They may feed on members of a range of taxonomically unrelated plant families (polyphagy); others may limit themselves to plants from one family, from closely related families (oligophagy) or from one genus or even species (monophagy). Historically, the central problem of interest has been to account for the degree of host specificity shown by phytophagous insects, the physiological basis of which is still largely unknown.

Verschaffelt (1910), Brues (1920), Dethier (1941,1954) and Fraenkel (1959) were among the earlier workers who recognized that chemical compounds play a major role in determining host specificity. These chemicals, acting as gustatory or olfactory stimuli, are detected by sensilla on various appendages such as the antennae, mouthparts and tarsi. The physiological basis of host plant selection can be best understood by a) studying the feeding behavior of the species; by b) isolating phytochemicals from the host plant, testing what compounds are responsible for feeding behavior and the behavioral responses that these compounds elicit; by c) ultrastructural studies of the chemosensilla involved and by d) analysing the neural activity of the chemoreceptor cells of sensilla that detect these chemicals.

Some early studies involving behavioral analyses and, occasionally, electrophysiological observations on the silkworm *Bombyx mori* (Ishikawa et al,1969), *Pieris brassicae* (David and Gardiner,1966), *Plutella macullipennis* (Nayar and Thorsteinson,1963), *Manduca sexta* and *Pieris brassicae* (Schoonhoven, 1969; Ma,1972), *Chrysolina brunsvicensis* (Rees,1969) and *Leptinotarsa decemlineata* (Stürckow and Löw, 1961, Bongers,1970) have provided evidence for a high degree of chemosensory specificity in these species. These studies stressed the role of single compounds in feeding behavior.

Schoonhoven (1967) described a chemoreceptive cell in *Pieris brassicae* that was sensitive to glucosinolates. Other pertinent examples include the response of the carrot fly, *Psila rosae* to propylbenzenes (Berüter and Städler,1971; Städler,1972; Guerin and Städler,1980) and of the cabbage root fly, *Delia brassicae* to isothiocyanates (Wallbank and Wheatley,1979; Finch and Skinner,1982). Thus, the neurological correlate for such 'token stimuli' was considered to be *labelled sensory lines* which were thought to be hard-wired channels responding to single classes of stimuli and resulting in specific behaviors. In the case of *L.decemlineata*, it was implied that alkaloids (tomatine, in particular) exert their inhibitory influence directly by acting on the chemosensory system.

However, a reinvestigation of *M.sexta* (Yammamoto and Fraenkel,1960, Howard,1977) and *L.decemlineata* (Mitchell and Harrison,1985) failed to find specific chemicals that promote or inhibit feeding respectively. Instead, *L.decemlineata* adults respond best to a specific blend of green leaf volatiles released by potato (Visser and Avé,1978; Visser et al,1979). Current concepts stress the integration of patterned sensory input and the perception of total plant "Gestalts" (Dethier, 1970,1973,1976,1982) as being the most important elements of host plant recognition. This view suggests that chemosensory neurons, are not highly specific but instead have broad and overlapping spectra (Schnieder,1969; Blaney,1975, van Drongelen *et al*,1978 and Dethier,1973,1974,1977). Thus, sensory quality in these systems is coded not by individual labelled lines (although these may, in some cases, be a factor) but by large populations of interacting neurons which produce unique 'across-fibre' (Pfaffman,1941; Erickson,1967) patterns of response.

The oligophagous feeding habit of the Colorado potato beetle, *L.decemlineata* has been much investigated. Behavioral aspects of host finding and food selection by the larvae have been studied in detail by Chin (1950) and de Wilde (1958). Jermy (1961) investigated adult preferences for acceptability of various solanaceous and non-solanaceous plants. Hsiao and Fraenkel (1968 a,b) studied the relationships between various plant chemicals and host specificity of this insect. Mitchell and Harrison (1984,1985) and Mitchell and Schoonhoven (1974) studied the role of amino acids and alkaloids. Hsiao (1978) considered geographic diversity of plant preference in this species while Visser (1979) provided an excellent account of the olfactory capabilities in adult beetles.

The present study was designed to investigate the role of palpi in host selection behavior of adult beetles. When a beetle arrives on the leaf surface, its maxillary palpi tap its leaf surface extensively while its labial palpi are more or less in continuous contact. It is thus probable that the palpi help in sensory identification of the host plant by recognizing the leaf surface waxes which are specific to a particular plant species. Based on this idea, the primary focus of this project was to determine the proportion of contact chemosensory and olfactory sensilla on the maxillary and labial palpi. Other goals were to analyse the components of leaf surface waxes extracted from potato leaves, test fractions for effect on feeding behavior and by ablation, measure some behavioral responses mediated by the palpi.

This thesis is organized into four chapters. Chapters II and III give a detailed account of gustatory and olfactory sensilla on the maxillary and labial palpi and the galea based on scanning electron microscope (SEM) and transmission electron microscope (TEM) observations. Chapter IV is a study of the chemical composition of leaf surface waxes of potato using Fourier transformation infra-red spectroscopy (FT-IR), gas chromatography (GC) and mass spectroscopy (MS) techniques. In addition, thin-layer chromatography (TLC) was employed to differentiate the major components of leaf surface waxes of five other solanaceous species : *S.elaeagnifolium, Datura stromonium,S.nigrum, L.esculentum,* and *N.tabacum* which had been used previously in behavioral studies. Chapter V summarizes the behavioral studies conducted. Bioassays were conducted with crude wax extracts and the different fractions were exposed to the beetles.

## REFERENCES

Berüter,J and Städler,E (1971) An oviposition stimulant for the carrot rust fly from carrot leaves. Zeit. für Naturforschung 26b:339-340.

Blaney,WM (1975) Behavioral and electrophysiological studies of taste discrimination by the maxillary palps of larvae of *Locusta migratoria*(L.). J.exp.Biol. 62:555-569.

Bongers,W (1970) Aspects of host-plant relationship of the Colorado beetle.Meded.Landbouwhogeschool Wageningen.70-10.

Brues,CT (1920) The selection of food plants by insects with special reference to lepidopterous larvae. Am.Nat. 54:313-332.

Chin,Chuh-Teh (1950) Studies on the physiological relations between the larvae of *Leptinotarsa decemlineata* Say and some solanaceous plants. Tijdschr.PlZiekt 56:1-88.

David,WAL and Gardiner,BOC (1966) Mustard oil glucosides as feeding stimulants for *Pieris brassicae* larvae in a semi-synthetic diet. Ent.exp.appl. 9:247-255.

Dethier,VG (1941) Chemical factors determining the choice of food plants by *Papilio* larvae. Amer.Nat. 75:61-73.

Dethier,VG (1954) Evolution of feeding preferences in phytophagous insects.Evolution.8:33-54.

Dethier,VG (1970) Chemical interactions between plants and insects. In : Chemical Ecology. E.Sondheimer and JB Simeone (eds.) Academic Press,NY.pp:83-102.

Dethier,VG (1973) Electrophysiological studies of gustation in lepidopterous larvae II. Taste spectra in relation to food-plant discrimination.J.Comp.Physiol. 82:103-134.

Dethier,VG (1974) The specificity of the labellar chemoreceptors of the blowfly and the response to natural foods. J.Insect Physiol. 20:1859-1869.

Dethier,VG (1976) The importance of stimulus patterns for host-plant recognition and acceptance. In : The host-plant in relation to insect behavior and reproduction. T.Jermy (ed.) Plenum Press,NY pp:67-70.

Dethier,VG (1977) Gustatory sensing of complex mixed stimuli by insects. In : Olfaction and Taste Vol. VI. J Le

Magnen and P Macleod (eds.). Information Retreival,London. pp:323-331.

Dethier,VG (1982) Mechanism of host-plant recognition. Ent.exp.appl.31:49-56.

de Wilde,J (1958) Host plant selection in the Colorado potato beetle larva (*Leptinotarsa decemlineata* Say). Ent. exp.appl. 1:14-22.

Erickson,RP (1967) Neural coding of taste quality. In : The chemical senses and nutrition. MR Kare and O Maller (eds.).pp: 313-317. John Hopkins Press, Baltimore.

Finch,S and Skinner,G (1982) Trapping cabbage root flies in traps baited with plant extracts and with natural and synthetic isothiocyanates. Ent.exp.appl. 31:133-139.

Fraenkel,G (1959) The raison d être of secondary plant substances. Science 129 : 1466-1470.

Guerin,PM and Städler,E (1980) Carrot fly olfaction : behavioral and electrophysiological investigations. In :Olfaction and Taste VII. H. van der Staare (ed.). IRL Press Ltd., London : pp 95.

Howard,GR (1977) Isolation of fractions from jimsonweed (*Datura stromonium*) and horsenettle (*Solanum carolinense*) which contain hostplant specificity factors for the tobacco hornworm (*Manduca sexta*). Ph.D thesis, N.Carolina State Univ., Raleigh.

Hsiao,T (1978) Host plant adaptations among geographic populations of the Colorado potato beetle. Ent. exp. appl. 24 : 237-247.

Hsiao T and Fraenkel,G (1968a)Selection and specificity of the Colorado potato beetle for solanaceous and non solanaceous plants.Ann. Entomol.Soc.Amer. 61:493-503.

Hsiao T and Fraenkel,G (1968b) The role of secondary plant substances in the food specificity of the Colorado potato beetle, *Leptinotarsa decemlineata*. Ann.Entomol. Soc.Amer. 61: 485-493.

Ishikawa,JH, Hvias,T and Arai,N (1969) Chemosensory basis of host plant selection in the silkworm. Ent. exp.appl. 12:544-554

Jermy,T (1961) On the nature of the oligophagy in *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Acta. Zool Acad. Sci. Hung. 7: 119-132.

Ma,WC (1972) Dynamics of feeding responses in *Pieris* *brassicae* Linn. as a function of chemosensory input : A behavioral, ultrastructural and electrophysiological study. Med. Landbouwhogesch. Wageningen:72-11: 1-162.

Mitchell,BK and Harrison,GD (1984) Characterization of galeal chemosensilla in the adult Colorado beetle, *Leptinotarsa decemlineata*. Physiol. Entomol. 9:: 49-56.

Mitchell,BK and Harrison,GD (1985) Effects of *Solanum* glycoalkaloids on chemosensilla in the Colorado potato beetle : A mechanism of feeding deterrence? J.Chem. Ecol. 11: 73-83.

Mitchell,BK and Schoonhoven,LM (1974) Taste receptors in Colorado beetle larvae. J. Insect Physiol. 20:1787-1793.

Nayar,JK and Thorsteinson,JA (1963) Further investigations into the chemical basis of insect-host relationships in an oligophagous insect, *Plutella macullipennis* (Curtis). Can.J.Zool. 41: 923-929.

Pfaffman,C (1941) Gustatory afferent impulses. J.Cell Comp. Physiol. 17: 243-258.

Rees,CJC (1969) Chemoreceptor specificity associated with choice of feeding site by the beetle, *Chrysolina brunsvicensis* on its foodplant, *Hypericum hirsutum*. Ent.exp.appl. 12: 565-584.

Schneider,D (1959) Insect olfaction : deciphering system for chemical messages. Science 163: 1031-1037.

Schoonhoven,LM (1967) Chemoreception of mustard oil glucosides in larvae of *Pieris brassicae*. Proc. Kon. Ned.Akad.Wtsch. C,70: 556-568.

Schoonhoven,LM (1969) Gustation and foodplant selection in some lepidopterous larvae. Ent.exp.appl. 12: 535-546.

Städler,E (1972) Über die Orientierung und das Wirtswahlverhalten der Möhrenfliege, *Psila rosae* F. (Diptera:Psilidae). II. Imagines. Zeit.Ang.Entomol. 70: 29-61.

Stürckow,B and Löw,I (1961) Die Wirkung einiger *Solanum* - alkaloidglykoside auf den Kartoffelkäfer, *Leptinotarsa decemlineata* Say. Ent. exp.appl. 4: 133-142.

van Drongelen,WA,Holley,A and Doving,KB (1978) Convergence in the olfactory system: Quantitative aspects of odor sensitivity. J.Theor.Biol. 71: 39-48.

Verschaffelt,E (1910) The cause determining the selection of food in some herbivorous insects. Proc. Roy. Acad. Amsterdam 13: 536-542.

Visser,JH and Avè,DA (1978) General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. Ent.exp.appl. 24: 738-749.

Visser,JH (1979) Leaf odour perception and olfactory orientation in the Colorado potato beetle. In Olfaction of the Colorado beetle at the onset of host plant selection. Ph.D thesis. Agricultural University, Wageningen. pp: 80-85.

Visser,JH, van Straten,S and Maarse,H (1979) Isolation and identification of volatiles in the foliage of potato *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. J.Chem.Ecol. 5:11-23.

Wallbank,BE and Wheatley,GA (1979) Some response of cabbage root fly (*Delia brassicae*) to ally isthiocyante and other volatile constituents of crucifers. Ann. Appl. Biol. 91: 1-12.

Yamamoto,RT and G Fraenkel,G (1960) Assay of the principal gustatory stimulant for the tobacco hornworm, *Protoparce sexta*, from Solanaceous plants. Ann.Entomol.Soc. Amer. 53:499-503.

## II. Ultrastructure of the sensory complex on the maxillary and labial palpi of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

### INTRODUCTION

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is an oligophagous pest restricted in its choice of food plants to the family Solanaceae. Studies at the population level have documented local adaptations to host plants in this species indicating that considerable specialization is possible (Hsiao,1974,1978,1981). It is generally assumed that chemosensilla are at least partly responsible for mediating host acceptance/rejection in insects and these have been studied in larval Lepidoptera of several species (Ishikawa,1965; Schoonhoven,1972; Schoonhoven and Dethier,1966; Ma,1972; Wieczorek,1976; De Boer,1985; De Boer *et al*,1977; Albert,1980). In the Colorado potato beetle, electrophysiological techniques have been used to investigate the function of antennal (Visser,1979) and galeal sensilla (Mitchell and Harrison,1984). To date palpal sensilla have largely been ignored, both in larval and adult beetles, except for the demonstration by Mitchell and Schoonhoven (1974) that larval maxillary and labial palpi are sensitive to the same amino acids as are the galeae.

The importance of maxillae in host-plant selection was first observed by Torii and Morii (1948) when they demonstrated that maxillectomized larvae of *Bombyx mori* fed on non-host plants. Waldbauer and Fraenkel (1961) and Waldbauer (1962) observed similar feeding on non host plants in larvae of tobacco hornworm, *Protoparce sexta* (now *Manduca sexta*). Since then, there have been a number of behavioral observations on the role of palpi in feeding by acridid grasshoppers (Haskell and Schoonhoven,1969; Haskell and Mordue,1969; Bernays and Chapman,1972; Bernays *et al*,1972). Klein (1982) provided an excellent account of sequences of palp movements with respect to individual behavioral patterns such as grooming, feeding and exploratory behavior in the cricket, *Gryllus bimaculatus*. In addition, palpal sensilla have been studied in locusts (Blaney and Chapman,1969; Blaney *et al*,1971 and Cook,1972); crickets (Klein,1981); cockroaches (Burry and Moran,1973; Altner,1975; Ramaswamy and Gupta,1981) and *Drosophila melanogaster* (Singh and Nayak,1985). Very little information is, however, available correlating ultrastructural observations with specific behavioral and electrophysiological data.

The present study was undertaken to determine the proportion of contact chemosensory and olfactory sensilla on the maxillary and labial palpi. This information will be useful in the design of future behavioral and electrophysiological experiments. It also provides data for comparison with analogous structures in orthopteroid insects.

## MATERIALS AND METHODS

Adults of *L.decemlineata* (Say) were collected in Edmonton and reared in the laboratory on leaves of greenhouse-grown potato, *Solanum tuberosum*.

For scanning electron microscopy (SEM), whole palpi were fixed in glutaraldehyde and refrigerated overnight. Subsequently, they were treated with protease solution (Dyer *et al*,1982) for 30 min, sonicated in Photoflo 200 for 30 secs, dehydrated in a series of ethanols, critical point dried and mounted on stubs. Some specimens were treated with tetrachloromethane for 30 secs which was then brought to a boil (Cuperus,1985). Fresh solvent was then added and boiled again. This was done four to five times.Such specimens were air-dried. All specimens were sputter coated with gold in a Nanotech Semprep 2 and observed using a Cambridge Stereoscan 250 electron microscope.

For transmission electron microscopy (TEM), palpal tips were fixed in chilled 5% glutaraldehyde in phosphate buffer. They were then placed in fresh, cold, glutaraldehyde fixative and refrigerated overnight. After fixation, palpal tips were washed twice in phosphate buffer, treated with 1% tannic acid in glutaraldehyde (Lai-Fook,1984) for 2 hr and post-fixed in 1% Millonig's (Millonig,1962) osmium tetroxide for 2 hr. Following post-fixation, specimens were washed twice in buffer, dehydrated through a graded series of ethanols and subsequently placed in propylene oxide for 1 hr with four changes. For better infiltration of plastic, the specimens were left overnight in a 1:1 solution of EPON 812 and propylene oxide. Specimens were transferred to fresh EPON for 2 hr after which they were transferred to embedding molds. Blocks were polymerized at $60^{0}$C for 15 hr. Sections were obtained using a Reichert OM U2 ultramicrotome and stained with ~~a saturated~~ solution of methanolic uranyl acetate for 20 min followed by lead citrate for 10 min. Sections were examined using a Philips EM300 electron microscope.

Distributions of sensilla on the maxillary and labial palpi were obtained by photographing segments of the palp tip at high magnifications and then reconstructing the apical tip. Five individuals were considered. Sensillar length and diameter (at the base) were measured on a minimum of five beetles. The data on numbers of different types of sensilla are averages of 10 palpal tips from 10 individual beetles.

## RESULTS

The paired maxillary (mp) and labial palpi (lp) which flank the mouthparts are 4 and 3 segmented respectively (Fig. 2.1). The maxillary palpi are about 900 µm in length while the labial palpi are 480-500 µm in length. An extensive field of sensilla is present on the membranous apical domes of the maxillary and labial palpi (Figs. 2.2,2.3). There are a total of 370 sensilla on the apices of the maxillary palpi and approximately 130 sensilla on those of the labial palpi. On the basis of SEM and TEM, the sensilla can be sorted into four distinct categories, three of which are common to both the maxillary and labial palpi.

### TYPE A

A 2.5 µm long and 1.8 µm wide sensillum, characterized by longitudinal grooves running from base to tip (Fig. 2.4). About 2 µm from the base, this sensillum narrows to about 0.6 µm where there is a depression around the entire circumference beyond which the grooves continue to the apex of the peg. There are a total of 170 sensilla of this type and they are unique to the maxillary palpi. These sensilla apparently have wall pores located in depressions between the ridges though none could be seen on the SEM. They have a thick wall with two concentric wall cylinders connected to each other by spoke canals (sc) and separated by pockets of uninnervated lumen (Fig. 2.8, ul). The inner cylinder contains dendrites (d)enclosed within a cuticular sleeve (Fig. 2.9, cs). Each sensillum is innervated by five or six bipolar neurons (Figs. 2.9,2.10), the dendrites of which are divided into proximal and distal dendritic segments by a constricted ciliary region. The dendrites do not branch based on examination of cross-sections around the ciliary region (Fig. 2.12).

The distal dendritic segments are enclosed by an electron dense tubular dendritic sheath (Fig. 2.11, ds). The sheath is open at each end and extends from the distal part of the ciliary region to the base of the peg. Irregular extensions of the wall of the sheath occur at either end. The distal dendritic segments originate from a funnel shaped ciliary collar (cc) seen at the region of greatest constriction in the dendrites (Fig. 2.13). Microtubules run parallel along the distal dendritic segments (Fig. 2.11) and at the periphery of the ciliary region, they reduce to a configuration of 9x2+0. The inner segment contains prominent mitochondria, vesicles and microtubules (Fig. 2.14). In addition, two basal bodies (bb) lie in tandem proximal to the constricted region, just beneath the ciliary region (Fig. 2.13). The distal basal body ~~is wider~~ than the proximal one and ciliary rootlets (cr) extend from both the basal bodies uniting proximally. Each dendrite continues proximally to an individual cell body containing an oblong nucleus and other cell organelles, viz., microtubules, mitochondria, golgi bodies and endoplasmic reticulum (cf.Fig. 2.35). Extending proximally from the cell body is an axon which joins the maxillary nerve.

There are five sheath cells associated with each of these sensilla (Figs. 2.13,2.15,2.35). The first, the inner sheath cell (in), encases the dendrites in the ciliary region, withdrawing to form an extracellular cavity, the ciliary sinus (Fig. 2.13, cs). It extends from the region of proximal termination of the dendritic sheath to the cell body. At the level of the ciliary sinus, the inner surface of this cell is microvillate and forms junctions with the proximal dendritic segments. Two intermediate sheath cells (i1, i2) encase the inner sheath cell (Figs. 2.13,2.15). They extend distally around the dendritic sheath to near the base of the peg and continue proximally alongside the cell body of the neuron. An outer sheath cell (ot) enwraps the distal extension of the intermediate sheath cell and terminates at the base of the peg. The outer and intermediate sheath cells elaborate extensive microvilli at the base of the peg and withdraw to form the sensillar sinus. The sensillar sinus is filled with a granular, electron dense material presumably secreted by the intermediate and outer sheath cells. Proximally, near the cell body of the neuron, a basal glial cell enwraps the cell body of the neurons and continues around its axons to the nerve. The cytoplasm of all the sheath cells contain numerous mitochondria along with other cell organelles such as golgi bodies, endoplasmic reticulum, vacuoles and microtubules.

## TYPE B

Type B sensilla are 4.5 µm long pegs and each is 2.8 µm wide at its base (Fig. 2.5). It is characterized by finger-like projections surrounding a pore at its apex. There are a total of 41 sensilla on the maxillary palpi and 58 on the labial palpi (Table 2.1).

These sensilla are innervated by six bipolar neurons (Figs. 2.17,2.18, d). A ciliary region divides dendrites into proximal and distal dendritic segments (Fig. 2.19). The ciliary region exhibits the typical characteristics seen in other insect sensilla. Microtubules are prominent in the distal dendritic segments which are enclosed within a dendritic sheath (ds). A typical 9x2+0 arrangement of microtubules is seen at the ciliary region.

Four sheath cells, the inner, intermediate, outer and basal sheath cells were observed associated with each of these sensilla (Figs. 2.18,2.19). The interrelationships of these cells is similar to those described for type A sensilla.

## TYPE C

There are no apparent pores on the tip of these sensilla although SEM observations indicate the presence of fine 'clefts' on the apical tip (Fig. 2.6). There are a total of 161 type C sensilla on the maxillary palpi and 72 on the labial palpi (Table 2.1). These sensilla are 4 µm long and 2.6 µm wide at the base.

TEM observations indicate that these sensilla are characterised by fine apical pores (p) which probably correspond to the 'clefts' seen on the SEM (Figs. 2.20,2.23). These sensilla are innervated by three dendrites which branch along the shaft of the peg into approximately 21 branches (Fig. 2.22, db). The branches appear to bead and vesicles are prominent (Fig. 2.21).

As in type B sensilla, a constricted ciliary region divides each dendrite into proximal (pd) and distal dendritic (dd) segments (Fig. 2.27). The dendritic sheath (ds), enclosing the distal segments, is convoluted and often isolates the dendrites (Fig. 2.25). In addition to microtubules, the distal segments have characteristic vesicles (v), some electron dense, which appear to originate from the region of the ciliary collar (Fig. 2.27).

Four sheath cells, as seen in type B, are associated with each of these sensilla (Figs. 2.27,2.36). However, it appears that the intermediate and outer sheath cells are associated with formation of two additional sinuses (s), in addition to the presence of the ciliary and sensillar sinuses (Figs. 2.28,2.29). From the region of the cell bodies to the proximal dendritic segments, a sinus cell extends within the intermediate sheath cell (Fig. 2.27). A sinus also exists within the outer sheath cell and opens into the sensillar sinus which is unusually large (Figs.2.24,2.26). In addition, the outer sheath cell maintains tight contact with the base of the peg (Fig. 2.24). The bipolar neurons possess a large nucleus enclosed by a thin layer of electron lucent cytoplasm and contain mitochondria, ribosomes and golgi elements (Fig. 2.30).

### TYPE D

A 5.0 µm long and 2.5 µm wide uniporous peg with a sunken socket. There are a total of 6 sensilla of this type on the maxillary palpi and 3 on the labial palpi (Fig. 2.7 and Table 1).

These sensilla are innervated by five bipolar neurons, four of which extend with their sensory processes to the tip of the peg (Fig. 2.33). The dendrite of the fifth neuron terminates at the hair base and has a typical tubular body (tb) with numerous parallel arranged microtubules. These sensilla have a sunken socket and possess a socket cylinder. Suspension fibres (sf) connect the cylinder with the base of the peg (Figs. 2.31,2.32). A joint membrane (jm) connects the socket and the basal end of the hair shaft. The ciliary region of the dendrites lies below the peg base. At the periphery of the ciliary region is a typical pattern of 9x2+0 microtubules (Fig. 2.34). The proximal and distal dendritic segments are similar in structure to those previously described for type B sensilla.

### COMPARATIVE MORPHOMETRY OF DENDRITES

The length of dendritic segments was reconstructed from several complete longitudinal sections from the different sensilla on the maxillary palpi (Fig. 2.37). They show that differences between sensillar types are much greater than individual variation. For example, the distance from the sensillum base to the dendritic cell bodies (cb) is about 58 µm in type C while it is about 96 µm in type A and 84 µm in type D. Also, the proximal dendritic segment (pd) in type C is short compared to that of type A.

## TOPOGRAPHY OF SENSILLA

The distribution of sensilla on the apical dome of the palpi shows no clear pattern and is summarized in Figs. 2.38 (a to g). Type A sensilla are distributed mainly on the apex of the oval dome of each maxillary palpus (Fig. 2.38a). Type B sensilla with finger-like projections at the tip, are interspersed throughout the sensillar field (Fig. 2.38b) whereas type C sensilla are distributed proximally and towards the periphery (Fig. 2.38c). Type D sensilla are distributed on the apices (Fig. 2.38d).

On the labial palpi, type B sensilla are distributed proximally on the apical tip (Fig. 2.38e) while type C are concentrated on its apex and on the lateral side (Fig. 2.38f). As in the maxillary palpi, type D sensilla occur on the periphery (Fig. 2.38g).

## DISCUSSION

Cuticular specializations of sensilla on the maxillary and labial palpi of adults of *L.decemlineata* are similar to those described in other insects (Hansen,1978; Altner and Prillinger,1980; Zacharuk,1980;1985). In insect sensilla, structure of the dendritic segments and their cuticular specialization can provide information on the modalities of stimuli (Altner and Prillinger,1980). Considering the types of sensilla present, it appears that palpi in *L.decemlineata* are capable of perceiving mechanical and chemical stimuli.

Porous sensilla in insects were first described in grasshoppers by Slifer *et al*,(1959) and since then, the presence of multiple pores has been a criterion for identification of olfactory sensilla. Type A sensilla are unique in this beetle due to the presence of spoke channels. Similar sensilla have been observed mostly in blood feeding insects (Bernard,1974; Levinson *et al*,1974; Steinbrecht and Müller,1976) but also in grasshoppers (Slifer *et al*,1959). It has been suggested that such sensilla respond to humidity and temperature in addition to being olfactory (Bernard,1974; Steinbrecht and Müller,1976). Recent evidence suggests that hygroreceptors are essentially non-porous (np) sensilla with an inflexible socket, while thermoreceptive sensilla have wall pores (Altner *et al*,1983). In a comprehensive study of antennal sensilla in 18 insect species from 9 orders, Altner *et al*, (1983) observed that such a sensillum has three sensory cells forming a 'triad' responding to cold, wetness and dryness. A model for the action of humidity on np-sensilla has been proposed for certain antennal sensilla in *Periplaneta americana* (Yokohari, 1978; 1981; Altner *et al*,1983) where the hygroreceptive cells are considered to be modified mechanoreceptors. In type A sensilla with spoke channels, stimulus conducting mechanisms should be similar to those described for olfactory sensilla with pore tubule systems (Steinbrecht,1973). The odor molecules pass through the groove channel by diffusion, then through the sensillar liquor before contacting the dendritic membrane. However, in pore tubule systems, efficiency is probably greater since the pore tubules contact the dendritic membrane directly.

Type B sensilla with apical finger-like projections are uniporous pegs and probably function as gustatory chemosensilla. In larvae of *L.decemlineata*, the maxillary and labial palpi have 16 and 11 'peg-like' sensilla respectively (Mitchell and Schoonhoven, 1974). These include type B sensilla ('pegs with villa-like structures, Fig. 1, cf.Mitchell and Schoonhoven,1974) and type C sensilla ('pegs with a broader base and a rounded tip', cf. Mitchell and Schoonhoven,1974) seen in adults. By placing a pipette over the tip of the palpus, single cell responses were evoked with a threshold of 1mM for GABA. No responses were obtained from the other compounds tested which included sodium chloride, sucrose, some amino acids and chlorogenic acid. The ultrastructural studies presented here suggest that the responses were evoked from the type B sensilla seen in adults, which have the characteristics typical of insect gustatory sensilla. In some studies, it has been shown that uniporous sensilla also respond to odors (Dethier,1972; Städler and Hanson,1975).

Judging by their ultrastructure, type C sensilla are olfactory receptors. Branching of dendrites and fine apical pores are characteristics of insect olfactory sensilla whereby they increase sensitivity (Steinbrecht,1969; Lewis,1970). Vesicles occur around the ciliary core and dendritic branches. Though not identified as a criterion for insect olfactory sensilla, such vesicles have been seen to be present within many insect olfactory sensilla (Slifer and Sekhon, 1964; Scott and Zacharuk,1971). It has been suggested that these vesicles have a nutritive function in transporting materials to the dendritic terminals (Slifer,1970; Scott and Zacharuk,1971). Ernst (1969) suggested that coalescence of vesicles in the distal dendritic segment isolates part of the dendrite which becomes a branch. Extracellular vesicles budded off from the dendritic terminals are involved in pore filament formation (Zacharuk,1971).

The tight membrane contacts between the outer sheath cell and the cuticle seen in type C sensilla have been observed in insect olfactory sensilla. As discussed by Keil (1982,1984) and Keil and Steinbrecht (1984), these contacts probably serve to provide electrical isolation between the sensillar sinus and the general subcuticular space. de Kramer *et al*,(1984) provided evidence to show that there is high resistance between the sensillar sinuses of neighboring sensilla. Such contacts may also serve to attach the whole sensillum unit to the cuticle and have been observed in mechanoreceptors on the thorax of *Calliphora* (Keil and Thurm, 1979) and on the cerci of *Acheta* (Keil and Steinbrecht,1984).

Type D sensilla with a sunken socket and an apical pore, show all the characteristics typical of insect gustatory sensilla. Their relative positions on the palpi and insertion mechanisms through a flexible surface membrane and suspensory fibres relates to conduction of mechanosensory stimulus to the dendritic tubular body.

Sheath cells observed in the different types of sensilla are similar to those reported for other insect sensilla. However, the outer and intermediate sheath cells in type A and C bear extensive microvilli in association with a large number of mitochondria. Such microvillate membrane layers are known to occur in other insect tissues such as midgut, malpighian tubules, rectum, rectal papillae and salivary glands and are involved in transport of ions and solutes (Berridge and Oschman,1972). A similar function has been proposed for the microvillate membrane system of the outer sheath cell in insect sensilla (Kuppers and Thurm,1979). Thurm (1970,1974) proposed a model for campaniform sensilla on the halteres of flies in which he suggested the presence of an electrogenic pump on the membrane system of the outer sheath cell involved in active transport of ions and which is capable of generating a large trans-epithelial voltage. Considering the enlarged membrane of the outer and intermediate sheath cells in types A and C sensilla, it is reasonable to assume that a continuous current flows during ion transport activity within a circuit comprising these cell membranes, that recycles the transported ions. Thus, structural relationships suggest that the intermediate sheath cell transports ions to and around the neuron through the inner sheath cell and that both the intermediate and outer sheath cells transport or secrete through their sinuses into the sensillar sinus.

Comparative morphometry of dendrites revealed striking differences in the dimensions of sheath cells among the various sensillar types on the palpi of *L.decemlineata*. The small size of these sensilla have so far prohibited electrophysiological recording which would help further in correlating structure and function in these sensilla.

The neuronal cell bodies of the different types of sensilla aggregate in the second palpal segment. The number of axons were not counted for the different sensillar types but on average, a 1:1 ratio of sensory dendrites to axons was observed. The axons had a beaded appearance in longitudinal section an appearance common in insect chemosensilla and also in crustacean chemoreceptors

(Ghiradella *et al*,1968), insect olfactory pegs (Slifer and Sekhon,1961; Steinbrecht,1969) and in ticks (Foelix and Chu-wang,1972).

The distribution of the various types of sensilla on the apical dome of the maxillary and labial palpi of *L.decemlineata* suggest that both gustatory and olfactory chemoreceptors are prominent. Since the antennae are involved in assessing olfactory cues from plants (Ma,1972; Visser, 1979), the large number of gustatory sensilla appears appropriate in view of the fact that the palpi contact surfaces prior to and during feeding by the insect. This also explains the behavioral sequence whereby the palpi tap the leaf surface. It has been suggested that olfactory neurons have a range of sensitivity to different plant compounds and that large numbers may facilitate discrimination at a distance (Kafka,1970). Altner and Stetter (1980), for example, observed 200 olfactory sensilla of four different types on the maxillary palpi of *P.americana*. However, these sensilla had characteristic responses to some alcohols and butyric acid but, unlike sensilla on the antennae, none of them responded to the sex pheromone of this insect.

It has been shown that adult beetles of *L.decemlineata* can distinguish potato odor from a distance (de Wilde *et al*,1969; Visser,1979) and that receptors in the terminal five antennal segments are responsible for such discrimination (Schanz,1953; de Wilde,1976). Ma and Visser

(1978) and Visser (1979) suggested that the olfactory system in adult beetles is very selective and concluded that 25 cells were representative of the sensillum population of the whole antenna and that the insect could distinguish between complex odors with this complement of cells. These cells were shown to respond to general green leaf volatiles and to respond best to trans-2-hexen-1-ol, cis-3-hexen-1-ol, hexanol-1, trans-2-hexannal, hexanal and cis-3-hexenylacetate.

This raises the question of the need for a high number of olfactory sensilla on the maxillary palpi. Presumably, once the adult beetles arrive on a potato leaf facilitated by action of their antennal olfactory sensilla, there probably arises a need for close range monitoring of host plant volatiles. It appears reasonable to speculate, that, at this stage of the behavioral sequence prior to feeding, the olfactory sensilla on the palps are able to detect low concentrations of primary alcohols, secondary alcohols and aldehydes present in the surface waxes of leaves. Based on observations by Mitchell and Schoonhoven (1974), I note the absence of type A olfactory sensilla in larvae of *L.decemlineata*, which further supports the importance of olfaction in host selection by adult beetles. Palpation observed in adults prior to taking a bite may serve to increase ventilation around the palpi and to enable successive reception of stimuli at different points in space thereby ensuring a high resolution of incoming stimuli. This information must be integrated with that from antennal olfactory sensilla. It has been shown in *P.americana* and *L.migratoria* that receptor cell axons from the antennal flagellum terminate in glomeruli of the ipsilateral deutocerebrum while the lobus glomeratus in the tritocerebrum receives input from the maxillary palpi and also processes from deutocerebral nerves (Ernst *et al.* 1977).

Surface waxes of host plants have been shown to be important in food choice by acridids where the number of sensilla are also reduced to a few types (Blaney and Chapman,1969;1970). It is thus probable that chemosensilla on the palpi perceive specific compounds in surface waxes.

In his extensive review, Chapman (1982) recognized several selection pressures likely to affect number of chemoreceptors, including insect size and the need for sensitivity. Although the numbers of chemoreceptors on the mouthparts of several species of locust and grasshopper varies in relation to body size (Chapman and Thomas, 1978), in *L.decemlineata*, there are no such obvious correlations based on observations on larvae by Chin (1950) and Mitchell and Schoonhoven (1974).

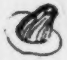
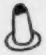
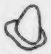
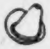
Chapman (1982) suggested that representatives of older taxa like the Orthoptera and Apterygota are less specialised and hence have more sensilla. According to him, this results in 'across-fibre' patterning of response in insects of this groups to give versatility to the system.

Endopterygotes, on the other hand, are specialised in their feeding habits because of their having tuned receptors responding to 'labelled-lines'. However, such sharply tuned receptors have been identified in only a few endopterygotes despite numerous claims for the presence of feeding stimulant/deterrent receptors. Conclusive electrophysiological evidence is available only for the pheromone receptor in males of *Bombyx mori* (Kaissling, 1971); glucosinolate sensitive cells in *Pieris brassicae* (Ma and Schoonhoven,1973) and *Mamestra brassicae* (Wieczorek,1976) and the hypericin sensitive cell in *Chrysolina brunsvicensis* (Rees,1969).

Food specialization within known species of Acridoidea are speculated to have arisen by " avoidance of unacceptable plants due to diversity of their secondary chemicals rather than as a positive adaptation to the chemical properties of the favoured food plants" (Chapman,1982). He even indicated that in such species, chemical adaptation to the host plant is not complete and that in the absence of the host, some of these species are known to accept other plants. In the species discussed, there is no reference to any data on numbers of sensilla on mouthparts or antennae.

My observations on *L.decemlineata* do not support the generalized observations by Chapman (1982). Numbers of palpal sensilla on adult *L.decemlineata* are high and do not fall within the generalized trend of fewer sensilla on specialized insects. Chapman's generalizations fail to explain relationships between sensilla on the antennae and the insect's size and/or taxonomic position. As the same insect is able to detect between different and simultaneously presented complex sources and as there is considerable overlap of compounds between these sources, peripheral coding cannot be assumed to occur by action of labelled lines alone but by a system of receptors with broad overlapping spectra. These mixtures are presumed to be coded according to an across-fibre pattern of response with each substance evoking a specific profile of relative levels of excitation in the cells. These 'generalist' cells are capable of fine grain sensory discrimination among the many complex stimuli present simultaneously in the environment of the insect.

Table 1 : Basic structural features of different sensilla on the palpi of *L.decemlineata*

| Type | Scheme | # of sensilla* |  | # of cells | Size (µm) |  |  | Wall thickness | Pores | Expected # of axons |  | Possible functions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  | MP | LP |  | Length | Width | Socket |  |  | MP | LP |  |
| A |  | 170±6 | -- | 5-6 | 2.5 | 1.8 | 4.8 | 0.5 | WP | 820-1050 | -- | Olfactory |
| B |  | 41±4 | 58±3 | 6 | 4.5 | 2.8 | 3.6 | 0.6 | AP | 246±24 | 348±18 | Gustatory |
| C |  | 161±6 | 72±4 | 3 | 4.0 | 2.6 | 5.5 | 0.8 | AP | 483±18 | 216±12 | Olfactory |
| D |  | 4±1 | 3±1 | 5 | 5.0 | 2.5 | 4.0 | 0.4 | AP | 20±5 | 15±5 | Gustatory |

* based on 10 observations
AP: apical pore; LP: labial palpi; MP: maxillary palpi; WP: wall pores

**Figs. 2.1-2.7 : SEM micrographs of sensilla on the maxillary and labial palpi of adult *L.decemlineata*.**

**Fig. 2.1** : Ventral view of mouthparts of *L.decemlineata*. ga=galea; lp=labial palp; md=mandible; mp=maxillary palp (Scale=400μm).

**Fig. 2.2** : Side view of the apical tip of maxillary palp (Scale=20μm).

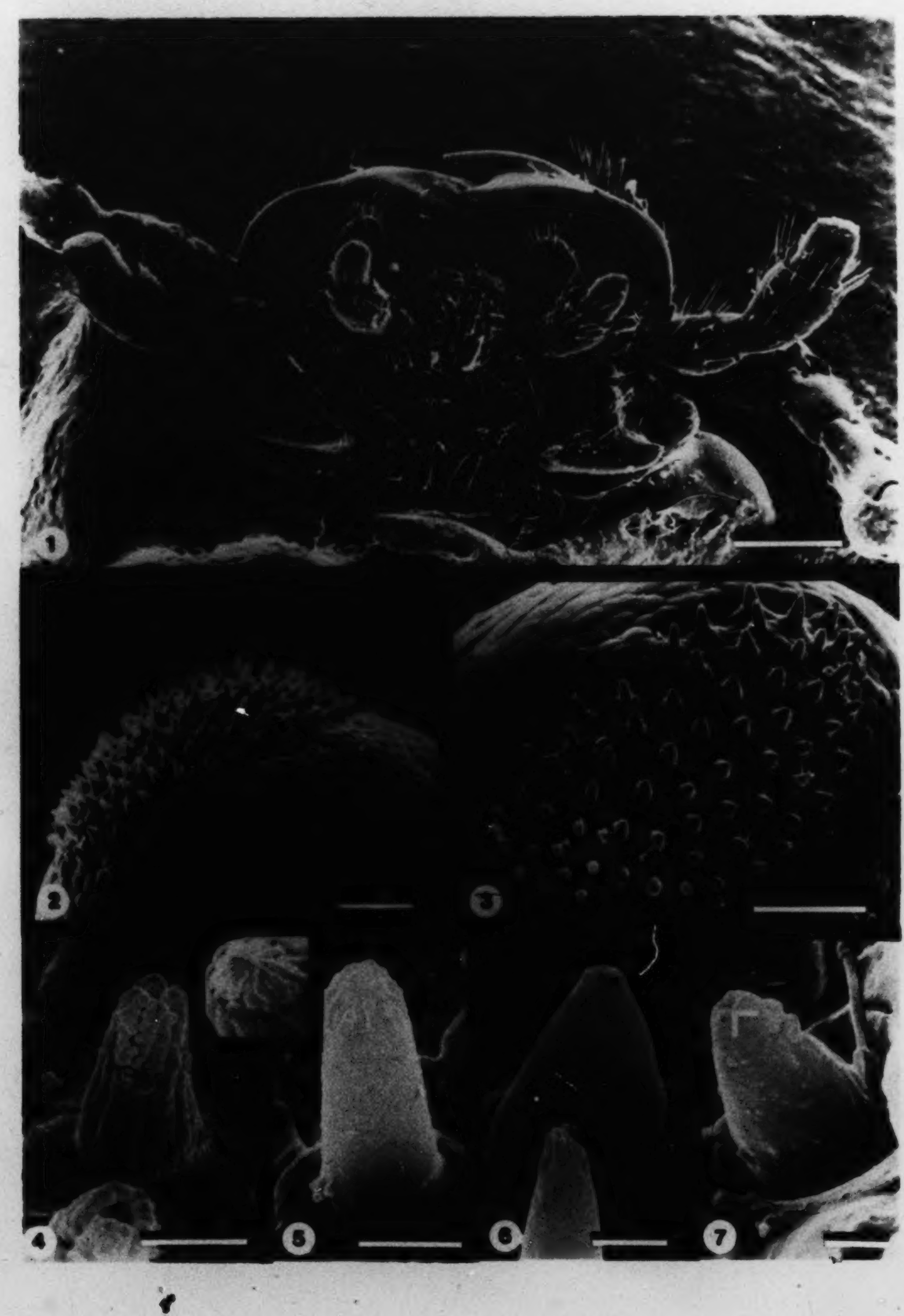
**Fig. 2.3** : Distribution of sensilla on the apical tip of the labial palp (Scale=20μm).

**Fig. 2.4** : Type A sensillum with longitudinal grooves (Scale=2μm). (Inset) Apical tip of type A sensillum showing depression along the circumference (Scale=same as in Fig.4).

**Fig. 2.5** : Type B sensillum with apical finger like projections (Scale=2μm).

**Fig. 2.6** : Type C sensillum. Note the fine indentations (arrows) on the tip of the sensillum (Scale=2μm).

**Fig. 2.7** : Type D sensillum with a single apical pore (Scale=1μm).


1
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**Figs. 2.8-2.15 : TEM sections of type A sensillum.**

**Fig. 2.8** : Cross-section through the peg of type A sensillum. Spoke canals (sc) extend from the dendritic lumen to the exterior. ul=uninnervated lumen (Scale=0.5µm).

**Fig. 2.9** : A thicker cross-section through the constricted area near the apical tip of type A sensillum showing the five dendrites (d) enclosed within a cuticular sleeve (cs) sl=sensillum liquor;ul=uninnervated lumen (Scale=0.5µm).

**Fig. 2.10** : Longitudinal section through the base of type A sensillum showing an enlarged sensillar sinus (ss) filled with a granular material. Note that the distal dendritic segments near the peg base are enclosed within a cuticular sleeve. Proximally, the dendrites (d) are enclosed within the dendritic sheath (ds) (Scale=2µm).

**Fig. 2.11** : Longitudinal section distal to the ciliary region in type A sensillum showing parallel arrangement of microtubules within a dendrite (d). ds=dendritic sheath; in=inner sheath cell;i1,i2=intermediate sheath cells;m=mitochondria;ot=outer sheath cell;ss=sensillar sinus. (Scale=2µm).

**Fig. 2.12** : Cross-section distal to the ciliary region in type A sensillum showing 6 dendrites. The dendritic sheath (ds) is petering out at this stage and portions of the sensory sinus are still visible (arrowheads). ds=dendritic sheath; in=inner sheath cell;i1,i2=intermediate sheath cells;ot=outer sheath cell(Scale=1µm).

**Fig. 2.13** : Longitudinal section at the ciliary region showing the distal and proximal dendritic segments with prominent basal bodies (bb) and ciliary rootlets (cr). cc=ciliary collar;dd=distal dendritic segments;ds=dendritic sheath; in=inner sheath cell;i1,i2=intermediate sheath cells; ot=outer sheath cell;pd=proximal dendritic segments. (Scale=1µm).

**Fig. 2.14** : Cross-section through the proximal dendritic segments in type A sensillum with prominent microtubules (arrows) within the dendrites (d). Septate junctions occur between the dendritic segments (arrowheads) in=inner sheath cell (Scale=0.5µm).

**Fig. 2.15** : Cross-section through type A sensillum proximal to the ciliary region. cs=ciliary sinus; d=dendrites; i1,i2=intermediate sheath cell; m=mitochondria (Scale=1µm).

**Figs. 2.16-2.19 : TEM sections through type B sensillum**

**Fig. 2.16** : Longitudinal section through type B sensillum with apical finger-like projections in the region of the pore (arrow). d=dendrites (Scale=4μm).

**Fig. 2.17** : Cross-section through the peg of type B sensillum showing the five dendrites (d) enclosed within a dendritic sheath (ds). (Scale=1μm).

**Fig. 2.18** : Cross-section through the distal dendritic segments (d) just distal to the ciliary region with the dendritic sheath (ds) petering out. in=inner sheath cell; it=intermediate sheath cell; ot=outer sheath cell.(Scale=1μm).

**Fig. 2.19** : Cross-section through different levels in the ciliary region of type B sensillum innervated by 6 dendrites, three containing the double arrangement of microtubules. cs=ciliary sinus; in=inner sheath cell; it=intermediate sheath cell (Scale=1μm).

**Figs. 2.20-2.23 : TEM sections through type C sensillum.**

**Fig. 2.20** : Cross-section through type C sensillum showing fine apical pores (p). (Scale=1μm).

**Fig. 2.21** : Longitudinal section through type C sensillum showing dendritic branches (db) with prominent vesicles (v). (Scale=2μm).

**Fig. 2.22** : Cross-section through type C sensillum showing dendritic branches (db). (Scale=1μm).

**Fig. 2.23** : Longitudinal section through type C sensillum showing fine apical pores (p). (Scale=1μm).

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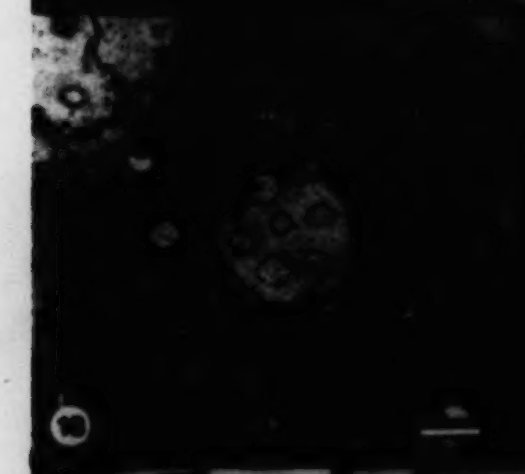
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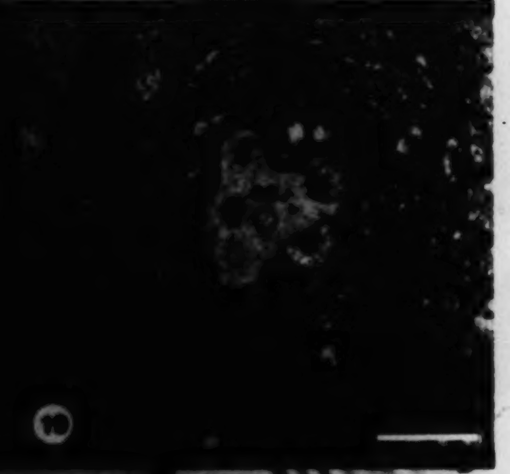
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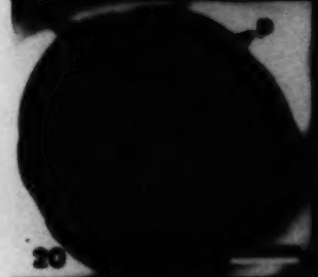

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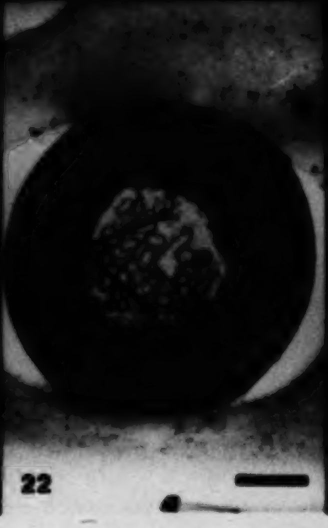
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**Figs. 2.24-2.29 : TEM sections through type C sensillum.**

**Fig. 2.24** : Longitudinal section through type C sensillum showing an enlarged sensillar sinus (ss). Note the tight membrane contacts (arrows) between the outer sheath cell (ot) and the sensillum base. d=dendrites. (Scale=4µm).

**Fig. 2.25** : Cross-section through type C sensillum innervated by three dendrites (d). Note the invaginations of the dendritic sheath (ds). in=inner sheath cell. (Scale=2µm).

**Fig. 2.26** : Cross-section through the distal dendritic segments of type C sensillum. Additional sinuses arising from the intermediate sheath cell (it) and outer sheath cell (ot) cell are characteristic at this level. in=inner sheath cell.(Scale=2µm).

**Fig. 2.27** : Longitudinal section through the ciliary sinus (cs) in type C sensillum. Electron dense and electron lucent vesicles (v) are seen within the ciliary collar (cr) in the distal dendritic branches (dd). bb=basal bodies; cr=ciliary rootlets; in=inner sheath cell; it=intermediate sheath cell; ot=outer sheath cell (Scale=2µm).

**Fig. 2.28** : Cross-section through the proximal dendritic segments (d) of type C sensilla. The inner sheath cell (in) is extensively lamellated at this level (arrowhead=cilium). cs=ciliary sinus; it=intermediate sheath cell; ot=outer sheath cell. (Scale=1µm).

**Fig. 2.29** : Cross-section through the proximal dendritic segments (d) of type C sensillum enclosed by a large sinus (s) arising from the basal glial sheath cell. in=inner sheath cell; it=intermediate sheath cell; v=vesicles. (Scale=1µm).

**Fig. 2.30** : Oblique section through the proximal region of a maxillary nerve branch showing axons (a) encompassed by a glial sheath cell (g) and surrounded by nucleus of epidermal cells. n=nucleus of the glial cell; nd=cell body of a dendrite. (Scale=2µm).

**Figs. 2.31-2.34 : TEM sections through type D sensillum.**

**Fig. 2.31** : Longitudinal section through type D sensillum with a sunken socket. The sensillum is attached to the socket cylinder by a joint membrane (jm) and suspension fibres (sf). sp=socket septum. (Scale=0.5µm).

**Fig. 2.32** : Oblique section through type D sensillum showing 4 dendrites (d) with one forming a tubular body (tb). ds=dendritic sheath. (Scale=1µm).

**Fig. 2.33** : Cross-section through the socket region in type D sensillum. c=socket collar; jm=joint membrane; ss=sensillum liquor; tb=tubular body.(Scale=1µm).

**Fig. 2.34** : Cross-section through the ciliary region in type D sensilla with three dendrites (d) showing the typical double microtubules while the mechanosensory dendrite is through the rootlets. cs=ciliary sinus; in=inner sheath cell; it=intermediate sheath cell; ot=outer sheath cell.(Scale=1µm).

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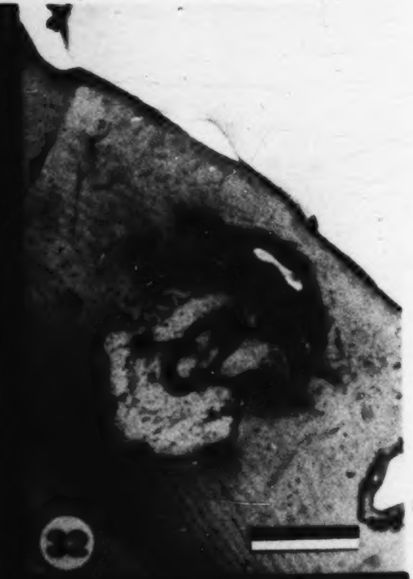
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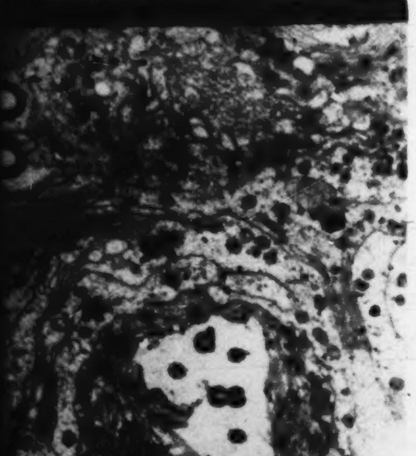
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**Fig. 2.35** : Montage of type A sensillum. bn=nucleus of basal sheath cell; cs=ciliary sinus; dd=distal dendritic segments; in=inner sheath cell; inn=nucleus of inner sheath cell; i1,i2=intermediate sheath cells; itn=nucleus of intermediate sheath cell; ot=outer sheath cell; otn=nucleus of outer sheath cell; pd= proximal dendrites; 1-5=dendritic cell bodies. (Scale=5µm).

**Fig. 2.36** : Montage of type C sensillum. in=inner sheath cell; inn=nucleus of inner sheath cell; it=intermediate sheath cell; itn=nucleus of intermediate sheath cell; ot=outer sheath cell; otn=nucleus of outer sheath cell; 1-3=dendritic cell bodies. (Scale=5μm).

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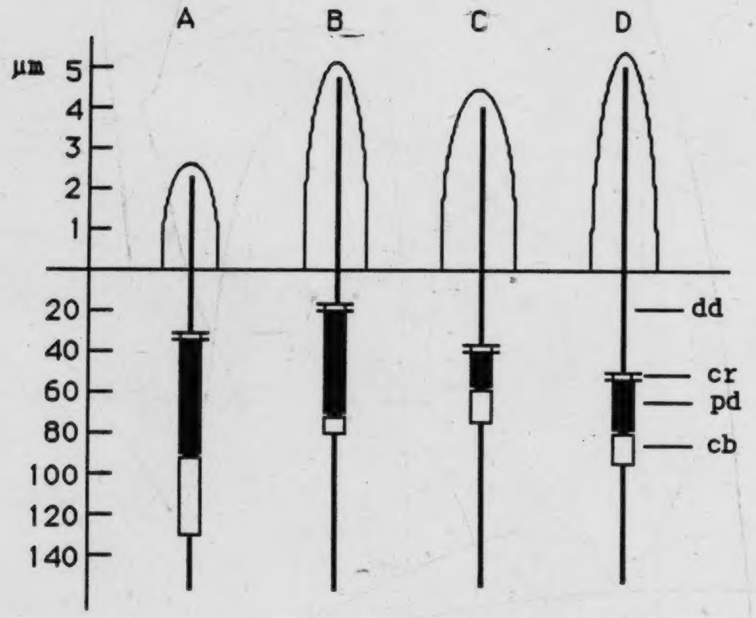
**Fig. 2.37** : Comparision of the length of dendritic segments in different chemosensillum types on the maxillary palpi of *L.decemlineata*. Only one dendrite per sensillum is considered. The values represent means from 5 sensilla. dd=distal dendritic segment; cb=cell bodies; cr=ciliary region; pd=proximal dendritic segment.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cb

**Fig. 2.38 a-g : Map of sensillar distribution on the maxillary and labial palpi.**

**Fig. 2.38a**: Distribution of type A sensillum on the maxillary palpi (Scale=20 µm).

**Fig. 2.38b**: Distribution of type B sensillum on the maxillary palpi.

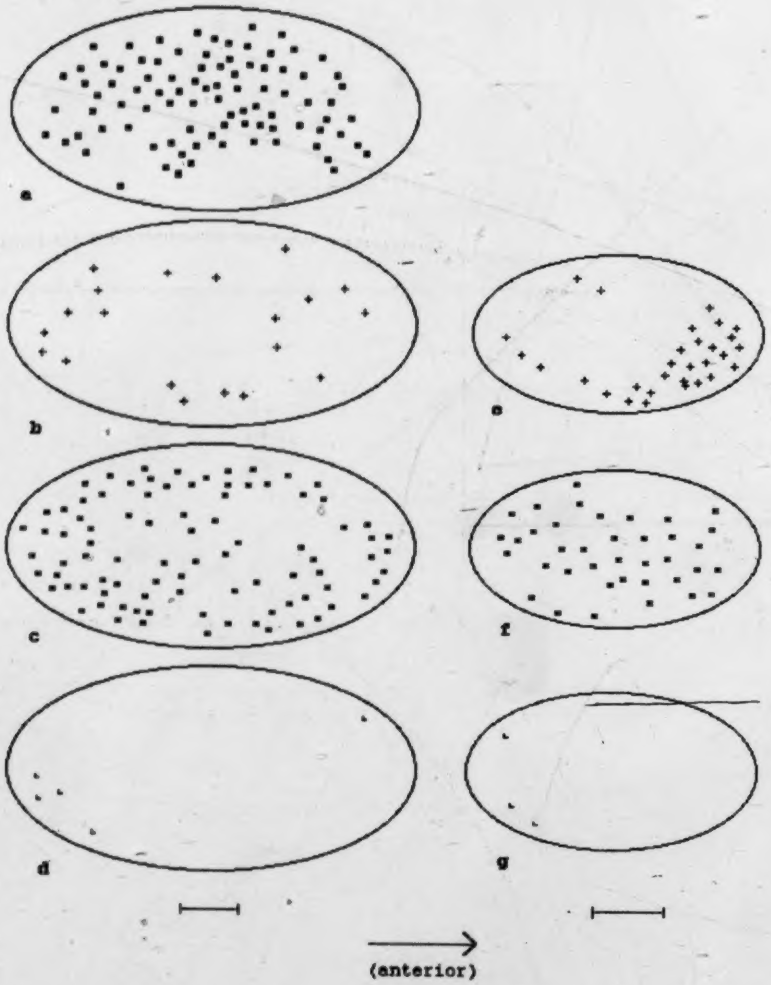
**Fig. 2.38c**: Distribution of type C sensillum on the maxillary palpi.

**Fig. 2.38d**: Distribution of type D sensillum on the maxillary palpi.

**Fig. 2.38e**: Distribution of type B sensillum on the labial palpi (Scale=20 µm).

**Fig. 2.38f**: Distribution of type C sensillum on the labial palpi.

**Fig. 2.38g**: Distribution of type D sensillum on the labial palpi.


a
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g
(anterior)

# REFERENCES

Albert,PJ (1980) Morphology and innervation of mouthpart sensilla in larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera:Tortricidae). Can.J.Zool.58: 842-851.

Altner,H (1975) The microfibre texture in a specialized plastic cuticle area within a sensillum field on the cockroach maxillary palp as revealed by freeze fracturing. Cell Tissue Res. 105: 79-88.

Altner,H and Prillinger,L (1980) Ultrastructure of invertebrate chemo-, thermo- and hygroreceptors and its functional significance. Int.Rev.Cytol.67: 69-139.

Altner,H and Stetter,H (1980) Olfactory input from the maxillary palps in the cockroach as compared with the antennal input. In Olfaction and Taste VII. London,Washington DC. Information Retreival:195.

Altner,H,Schaller-Selzer,L,Stetter,H and Wohlrab,I (1983) Poreless sensilla with inflexible sockets : A comparative study of a fundamental type of insect sensilla probably comprising thermo and hygroreceptors. Cell Tissue Res.234: 279-307.

Bernard,J (1974) Etude electrophysiologique de recepteurs impliques dans l'orientation vers e'hote et dans l'acte hematophage chez un Hemiptere : *Triatoma infestans*. Thesis, University of Rennes.

Bernays,EA and Chapman,RF (1972) The control of changes in peripheral sensilla associated with feeding in *Locusta migratoria*. J.exp.Biol. 57: 755-763.

Bernays,EA, Blaney,WM and Chapman,RF (1972) Changes in chemoreceptor sensilla on the maxillary palps of *Locusta migratoria* in relation to feeding. J.exp.Biol. 57: 745-753.

Berridge,MJ and Oschman,JL (1972) Transporting epithelia. Academic Press. London.

Blaney,WM and Chapman,RF (1969) The anatomy and histology of the maxillary palp of *Schistocerca gregaria* (Orthoptera, Acrididae).J.Zool.London 157: 509-535.

Blaney,WM and Chapman,RF (1970) The functions of the maxillary palps of Acrididae (Orthoptera). Ent.exp.appl. 13: 363-376.

Blaney,WM,Chapman,RF and AG Cook (1971) The structure of the terminal sensilla on the maxillary palps of *Locusta migratoria* (L.) and changes associated with moulting. Z.Zellforsch.121: 48-68.

Burry,RW and Moran,DT (1973) Sense organs in the cockroach maxillary palp. Naturwissenschaften 60: 521.

Chapman,RF (1982) Chemoreception : The significance of receptor numbers. Adv.Insect Physiol.16: 247-356.

Chapman,RF and Thomas,JG (1978) The number and distribution of sensilla on the mouthparts of Acridoidea. Acrida 7: 115-148.

Chin,Chuh-Teh (1950) Studies on the physiological relations between the larvae of *Leptinotarsa decemlineata* Say and some solanaceous plants. Tijdschr.PlZiekt.56: 1-88.

Cook,AG (1972) The ultrastructure of the A1 sensilla on the posterior surface of the clypeo-labrum of *Locusta migratoria migratorioides* (R & F). Z.Zellforsch. Mikr. Anat. 134: 539-554.

Cuperus,PL (1985) Inventory of pores in antennal sensilla of *Yponomeuta* spp. (Lepidoptera:Yponomeutidae) and *Adoxophyes orana* F.v.R. (Lepidoptera:Tortricidae). Int.J.Insect Morphol.& Embryol. 14(6): 347-359.

De Boer,G (1985) The role of phytochemicals, chemosensory organs,and feeding experience in food plant selection by the larvae of the tobacco hornworm, *Manduca sexta* (Lepidoptera:Sphingidae). Ph.D Dissertation; University of Maryland, Baltimore, USA.

De Boer,G, Dethier,VG and LM Schoonhoven (1977) Chemoreceptors in the preoral cavity of the tobacco hornworm, *Manduca sexta* and their possible function in feeding behavior. Ent.exp.appl. 21: 287-298.

Dethier,VG (1972) Sensitivity of the contact chemoreceptors of the blowfly to vapours. Proc.Natl.Acad.Sci.USA. 69: 2189-2192.

Dyer,LJ, Seabrook,WD and Jaeger,VA (1982) Preparation of insect contact chemosensilla for scanning electron microscopy. Can.Entomol.114: 891-896.

Ernst,K.-D (1969) Die Feinstruktur von Riechsensillen auf der Antenne des Aaskäfers *Necrophorus* (Coleoptera). Z.Zellforsch. Mikr. Anat. 94: 72-102.

Ernst,K.-D, Boeckh,J and Boeckh,V (1977) A neuroanatomical study on the organization of the central antennal pathway in insects. II.Deutocerebral connections in

*Locusta migratoria* and *Periplaneta americana*. Cell Tissue Res.176: 285-308.

Foelix,RF and Chu-Wang,I-Wu (1972) Fine structural analyses of palpal receptors in the tick *Amblyomma americanum* (L.). Z.Zellforsch. 129: 548-560.

Ghiradella,HT, Case,JF and Cronshaw,J (1968) Structure of aesthetascs in selected marine and terrestrial decapods. Chemoreceptor morphology and environment. Amer. Zoologist. 8: 603-621.

Hansen,K (1978) Insect chemoreception. In Hazelbauer GL(ed.) Taxis and behavior. Series B.Vol.5 New York. John Wiley and Sons. pp: 233-292.

Haskell,PT and Mordue,AJ (1969) The role of mouthpart receptors in the feeding behaviour of *Schistocerca gregaria*. Ent.exp.appl.12: 591-610.

Haskell,PT and Schoonhoven,LM (1969) The function of certain mouthpart receptors in relation to feeding in *Schistocerca gregaria* and *Locusta migratoria migratorioides*. Ent.exp.appl.12: 423-440.

Hsiao,T (1974) Chemical influence on feeding behavior of *Leptinotarsa* beetles. In L.Barton Browne (ed.). Experimental analyses of insect behavior. Springer.Berlin. pp: 237-248.

Hsiao,T (1978) Host plant adaptations among geographic populations of the Colorado potato beetle. Ent.exp.appl. 24: 237-247.

Hsiao,T (1981) Ecophysiological adaptations among geographic populations of the Colorado potato beetle in North America. In J.H.Lashomb and R.Casagrande (eds.): Advances in potato pest management. Pennsylvania. Hutchinson Ross.pp: 69-85.

Ishikawa,S (1965) Maxillary chemoreceptors in the silkworm. In T.Hayashi (ed.).Proc Int Symp Olfaction and Taste II. Pergamon Press, Oxford.

Kafka,WA (1970) Molekulare Wechselwirkungen bei der Ernegung einzelner Reichzeller.Z.vergl.Physiol. 70: 105-143.

Kaissling,KE (1971) Insect olfaction . In : Handbook of sensory physiology. L.M.Beidler (ed.). Vol. 4. Springer Verlag, Berlin

Keil,TA (1982) Contacts of pore tubules and sensory dendrites in antennal chemosensilla of a silkmoth : demonstration of a possible pathway for olfactory molecules. Tissue and Cell.14(3): 451-462.

Keil,TA (1984) Very tight contact of tormogen cell membrane and sensillum cuticle : Ultrastructural basis for high electrical resistance between receptor-lymph and subcuticular spaces in silkmoth olfactory hairs. Tissue And Cell 16(1): 131-135.

Keil,TA and Steinbrecht,RA (1984) Mechanosensitive and olfactory sensilla of insects. In : Insect ultrastructure (eds.) R.C.King and H.Akai) Vol.2. Plenum Press,New York.pp: 477-516.

Keil,TA and Thurm,U (1979) Die Verteilung von Membrankontakten und Diffusionbarrieren in epiderm. Sinnesorganen von Insekten. Verh.Dt.Zool.Ges. 72: 285.

Klein,U (1981) Sensilla of the cricket palp. Fine structure and spatial organization. Cell Tissue Res. 219: 229-252.

Klein,U (1982) The articulation of cricket palps : morphology and movement patterns in behavior. Physiol. Entomol. 7: 297-314.

Kuppers,J and Thurm,U (1979) Active ion transport by a sensory epithelium.I.Transepithelial short circuit current, potential difference, and their dependence on metabolism. J.Comp.Physiol.134: 131-136.

Lai-Fook,J (1984) Lamellar bodies in differentiating insect tissues during basal lamina formation as revealed by tannic acid.Tissue and Cell 16(6): 909-916.

Levinson,HZ, Levinson,AR, Müller,B and Steinbrecht,RA (1974) Structure of sensilla, olfactory perception and behaviour of the bed bug, *Cimex lectularius*, in response to its alarm pheromone. J.Insect Physiol. 20: 1231-1248.

Lewis,CT (1970) Structure and function in some external receptors. Symp.R.Entomol.Lond.5: 59-76.

Ma,WC (1972) Dynamics of feeding responses in *Pieris brassicae* Linn. as function of chemosensory input : a behavioral, ultrastructural and electrophysiological study. Meded. Landbouw Wageningen 72: 72-11.

Ma, WC and Schoonhoven,LM (1973) Tarsal contact chemosensory hairs of the large white butterfly *Pieris brassicae* and their possible role in oviposition behavior. Ent.exp.appl. 16:343-357.

Ma,WC and Visser,JH (1978) Single unit analyses of odour quality coding by the olfactory antennal receptor system of the Colorado beetle. Ent.exp.appl. 24: 520-533.

Millonig,GG (1962) Further observations on a phosphate buffer for osmium solutions in fixation.In S.S.Breese (ed.). Proc.5th. Int.Congr.on Electron microscopy Vol.II. New York.Academic Press.pp: 8.

Mitchell,BK and Harrison,GD (1984) Characterization of galeal chemosensilla in the adult Colorado beetle, *Leptinotarsa decemlineata*. Physiol. Entomol.9: 49-56.

Mitchell,BK and Schoonhoven,LM (1974) Taste receptors in Colorado beetle larva. J.Insect Physiol. 20: 255-264.

Ramaswamy,SB and Gupta,AP (1981) Sensilla on the antenna and the labial and maxillary palps of *Blatella germanica* (L.)(Dictyoptera:Blatellidae): their classification and distribution. J.Morph. 168: 269-279.

Rees,CJC (1969) Chemoreceptor specificity associated with choice of food site by the beetle, *Chrysolina brunsvicensis* on its foodplant, *Hypericum hirsutum*. Ent.exp.appl. 12: 565-583.

Schanz,M (1953) Der Geruchssinn des Kartoffelkafers (*Leptinotarsa decemlineata* Say).Z.vergl.Physiol. 35: 353-379.

Schoonhoven,LM (1972) Plant recognition by lepidopterous larvae.In : H.F.van Embden (ed.). Symp.R. Entomol. Soc. Lond.: 87-89.

Schoonhoven,LM and Dethier,VG (1966) Sensory aspects of host plant discrimination by lepidopterous larvae. Arch. Neerl.Zool. 16: 497-530.

Scott,DA and Zacharuk,RY (1971) Fine structure of the dendritic junction body region of the antennal sensory cone in a larval elaterid (Coleoptera). Can.J.Zool. 49: 817-821.

Singh,RN and Nayak,SV (1985) Fine structure and primary sensory projections of sensilla on the maxillary palp of *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae). Int.J.Insect Morphol. & Embryol. 14(5): 291-306.

Slifer,EH (1970) The structure of arthropod chemoreceptors. Ann.Rev.Ent.15: 121-142.

Slifer,EH, Prestage,JJ and Beams,HW (1959) The chemoreceptors and other sense organs on the antennal flagellum of the grasshopper (Orthoptera:Acrididae). J.Morph.105: 145-191.

Slifer,EH and Sekhon,SS (1961) Fine structure of the sense organs on the antennal flagellum of the honey bee, *Apis mellifera* Linnaeus. J.Morph. 109: 351-381.

Slifer,EH and Sekhon,SS (1964) The dendrites of the thin-walled olfactory pegs of the grasshopper (Orthoptera, Acrididae). J.Morph. 114: 393-410.

Städler,E and Hanson,FE (1975) Olfactory capabilities of the 'gustatory' chemoreceptors of the tobacco hornworm larvae. J.Comp.Physiol.104: 97-102.

Steinbrecht,RA (1969) Comparative morphology of olfactory receptors. In C.Pfaffmann(ed.) Olfaction and Taste III. New York Rockfeller University Press.pp: 3-21.

Steinbrecht,RA (1973) Die feinbau Olfaktorischer Sensillen des Seidenspinners (Insecta:Lepidoptera). Z.Zellforsch. 139: 533-565.

Steinbrecht,RA and Müller,B (1976) Fine structure of the antennal receptors of the bed bug, *Cimex lectularius* L. Tissue & Cell.8: 615-636.

Thurm,U (1970) Untersuchungen zur funktionellen Organization sensorischer zellverbände.Verh.Dtsch.Zool.Ges.Köln '70. Stuttgart-Fischer.pp: 79-87.

Thurm,U (1974) Mechanisms of electrical membrane responses in sensory receptors, illustrated by mechanoreceptors. In L.Jaenicke(ed.):Biochemistry of sensory functions. Berlin Springer.pp:367-390.

Torii,K and Morii,K (1948) Studies on the feeding habits of silkworm. Bull Res. Inst. Sericult.Sci. 2:6-12.

Visser,JH (1979) Electroantennogram responses of the Colorado beetle, *Leptinotarsa decemlineata*, to plant volatiles. Ent.exp.appl.25: 86-97.

Waldbauer,GP (1962) The consumption, digestion and utilization of Solanaceous and non solanaceous plants by larvae of the tobacco hornworm, *Protoparce sexta* (Johan.) (Lepidoptera:Sphingidae). Ent.exp.appl. 7: 253-269.

Waldbauer,GP and Fraenkel,G (1961) Feeding on normally rejected plants by maxillectomized larvae of the tobacco hornworm,*Protoparce sexta* (Lepidoptera: Sphingidae). Ann.ent.Soc.Am. 54: 477-485.

Wieczorek,H (1976) The glycoside receptor of the larvae of *Mamestra brassicae* L. (Lepidoptera:Noctuidae). J.Comp. Physiol. 106: 153-176.

de Kramer,JJ, Kaissling,K.-E and Keil,T (1984) Passive electrical properties of insect olfactory sensilla may produce the biphasic shape of spikes. Chem.Senses 8: 289-295.

Wilde,J de (1976) The olfactory component in host plant selection in the adult Colorado beetle (*Leptinotarsa decemlineata* Say). Symp. Biol. Hung. 16: 291-300.

Wilde,J de, Lambers-Suverkropp,K Hille Ris and Van Tol,A (1969) Responses to air flow and airborne plant odor in the Colorado beetle. Neth.J.Pl.Path.75: 53-57.

Yokohari,F (1978) Hygroreceptor mechanism in the antennae of the cockroach *Periplaneta*. J.Comp.Physiol.124: 53-60.

Yokohari,F (1981) The sensillum capitulum, an antennal hygro- and thermoreceptive sensilla of the cockroach *Periplaneta americana* L. Cell Tissue Res. 216: 525-543.

Zacharuk,RY (1971) Fine structure of peripheral terminations in the porous sensillar cone of larvae of *Ctenicera destructor* (Brown)(Coleoptera:Elateridae) and probable fixation artifacts. Can.J.Zool. 49: 789-799.

Zacharuk,RY (1980) Ultrastructure and function of insect chemosensilla. Ann.Rev.Entomol.25: 27-47.

Zacharuk,RY (1985) Antennae and sensilla. In G.A.Kerkut and L.I.Gilbert (eds.) : Comprehensive Insect physiology, biochemistry and pharmacology. Vol.6. Oxford.Permagon Press.pp: 1-69.

# III. Ultrastructure of the galeal sensory complex in adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

## INTRODUCTION

Sensory mechanisms underlying feeding behavior and host plant selection by phytophagous insects have been given considerable attention in recent years. Extensive studies have been conducted with larval Lepidoptera (Ishikawa,1965; Schoonhoven and Dethier,1966; Schoonhoven,1972; Ma,1972; Wieczorek,1976; De Boer,1985; De Boer *et al*, 1977; Albert, 1980); and with locusts (Blaney and Chapman,1969,1970; Blaney *et al*,1971); crickets (Klein,1981; Klein and Muller,1978) and mosquitoes (McIver,1982).

Galeal sensilla in chrysomelid beetles respond to nutrients and to secondary plant compounds. Mitchell and Gregory (1979) studied the physiology of the lateral galeal sensillum in larvae of the red turnip beetle, *Entomoscelis americana* and described responses to NaCl, glucosinolates and glucosides, while Sutcliffe and Mitchell (1980) described the response characteristics of analogous adult sensilla. Similar electrophysiological studies have been conducted on the galeae of larvae and adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera : Chrysomelidae) (Mitchell,1974,1985; Mitchell and Schoonhoven,1974; Mitchell and Harrison,1984,1985). Approximately 15 chemosensilla, which are not distinguishable on the basis of their external structure, beetle. All these sensilla are sensitive to sucrose, while only a few respond to L-alanine and gamma amino butyric acid (GABA). One of them, the α-sensillum, is much more sensitive to the amino acids than the others, and has a higher conductivity (Mitchell and Harrison, 1984). In addition, the same sensory cell predominates in the α-sensillum responses to amino acids and to sucrose.

In the present study, attempts were made to find differences in ultrastructure among galeal sensilla which correlate with known physiological differences.

## MATERIALS AND METHODS

Adults of *Leptinotarsa decemlineata* were collected in Edmonton and reared in the laboratory on leaves of greenhouse-grown *Solanum tuberosum* .

For scanning electron microscopy (SEM), specimens were prepared by air-drying excised heads and mounting them on stubs. Mounted stubs were gold coated in a sputter coater and observed using a Cambridge Stereoscan 250 electron microscope. To identify the $\alpha$-sensillum, specimens were prepared as outlined in Mitchell and Harrison (1984).

For transmission electron microscopy (TEM), galeal tips were dissected from live beetles and immersed in chilled 5% glutaraldehyde fixative in phosphate buffer. They were then placed in fresh, cold glutaraldehyde fixative and refrigerated overnight. After fixation, galeal tips were washed twice in phosphate buffer and post fixed in 1% Millonig's osmium tetroxide for 2h. After fixation, specimens were washed twice in buffer, dehydrated through a series of ethanol,and subsequently placed in propylene oxide for 1h with four changes. For better infiltration of plastic, the specimens were left overnight in a 1:1 solution of EPON 812 and propylene oxide. Specimens were transferred to fresh EPON for 2h after which they were transferred to embedding moulds. Blocks were polymerised at 60 $^0$C for 15h. Sections were obtained using a Reichert Om U2 ultramicrotome and stained with a saturated solution of methanolic uranyl acetate for 20 min followed by lead citrate for 10 min.

Sections were examined using a Philips EM300 electron microscope.

# RESULTS AND DISCUSSION

The galea (ga) in the adult Colorado potato beetle arise between the lateral, 4-segmented palpus and a medial, spoon-shaped fringed lacinia (Fig. 3.1). The galeae have mechanosensory hairs as well as chemosensitive apical pegs at their tips (Fig. 3.2). The mechanosensory hairs are more numerous in *Leptinotarsa decemlineata* than in the red turnip beetle, *Entomoscelis americana* and often obscure the chemosensitive pegs.

There are 11 to 15 apical pegs arranged in an irregular fashion on the tip of the galea (Fig. 3.2). These are protected by numerous mechanosensory hairs on the dorsal side and by fewer on the ventral side. Each chemosensory peg is uniporous, cylindrical and has a simple socket.

## APICAL PEGS

TEM studies indicate that the apical pegs are innervated by 5 neurons, 4 of which extend their sensory processes up to the tip of the peg (Fig. 3.3). The dendrite of the fifth neuron terminates at the hair base and exhibits a typical tubular body (Fig. 3.4, tb). However, one of the apical pegs, the α-sensillum, has 4 cells (3 chemosensitive plus 1 mechanosensitive) (Fig. 3.5). The α-sensillum is located approximately in the centre of the sensillar field and is surrounded ventrolaterally by 4 chemosensory pegs. Twenty-five galea were sectioned and in each one, only a single apical peg had 4 dendrites. The walls of the apical pegs are thick with a large lumen containing the dendrites

(Fig. 3.6). The dendrites are packed tightly and often present a telescoped appearance which is, in fact, an artifact of fixation, and is usually not evident when freeze substitution is employed (Steinbrecht,1980). The proximal dendritic segment is enclosed by an inner sheath cell (in) and the latter by an intermediate sheath cell (it) (Figs. 3.9, 3.10). There are a number of well developed longitudinal junctions between the dendritic membrane and the inner sheath cell (Fig. 3.9). These junctions are complexes of microfibrils and are assumed to be skeletal structures forming a protective barrier around the ciliary sinus and the dendritic segments within. The proximal dendritic segments have a typical microtubular arrangement of 9x2+0 (Figs. 3.7,3.8). At this point, the sheath cell encloses the ciliary sinus.

The presence of an apical pore and electrophysiological evidence (Mitchell and Harrison,1984) indicates that apical pegs are contact chemosensilla. In sections, the sensillum containing 4 cells was surrounded by other apical pegs, as indicated by SEM. Though its exact location could not be determined due to irregular arrangement of sensilla, the general position of the 4-cell sensillum is not inconsistent with that of the $\alpha$-sensillum as identified using electrophysiological and SEM techniques (Mitchell and Harrison, 1984). On the basis of this indirect evidence, we suggest that the $\alpha$-sensillum, which responds best to L-alanine and GABA, contains only 3 chemosensitive cells. It has been suggested that the unique response of the α-sensillum to 2 amino acids, and to mixtures of amino acids and sucrose, is probably due to a single cell; assuming that these compounds do not inhibit each other's activity. The physiological differences observed between the α-sensillum and the remaining apical pegs appear to have a morphological correlate in the reduced number of chemosensitive cells in the apical peg. It is likely that the sugar sensitive cell in these sensilla has secondarily become sensitive to the 2 amino acids. Neighbouring sensilla are moderately to poorly sensitive to the amino acids while retaining their sucrose sensitivity (Mitchell and Harrison, 1984). Why this should result with the loss of a sensory cell is not clear. It would be interesting to compare several species of *Leptinotarsa* using electrophysiological and TEM techniques. According to behavioral studies, larvae of several species are not equally sensitive to amino acids as feeding stimulants (Hsiao, 1974).

## APICAL HAIRS

In addition to the pegs, another type of sensillum occurs on the galeal tip. These hairs are shorter than the apical pegs and the pore at the tip is surrounded by finger-like projections (Fig. 3.12). There are 2 hairs on each galea (Fig. 3.11). At the base, 2 dendrites enter the hair along with an extension of the sensillar sinus, the sensillar lumen (Fig. 3.13, sl). The wall of this sensillum is thick and the lumen is just large enough to contain the dendrites. There is apparently no dendritic sheath surrounding the dendrites at this level. The sensillar lumen is filled with an electron dense material suggesting the presence of a fluid *in vivo*. At the base of the hair, the 2 channels (sensillar lumen and dendritic lumen) merge with the dendrites being surrounded by a dendritic sheath (Fig. 3.14, ds).

It is difficult to draw firm conclusions about the apical hairs. The absence of a tubular body does not necessarily rule out mechanosensitivity. Rice *et al* (1973) described a mechanosensillum in tsetse flies lacking a tubular body. Such a function was also ascribed to a club-shaped aporous sensillum in nymphs of *Schistocerca gregaria* (Bernays *et al*,1976). Nevertheless, the fact that the dendrites reach the tip of the hair makes it difficult to conclude that these hairs are mechanosensitive. The absence of a cuticular sheath within the hair shaft has been observed in several sensilla (Kaissling,1971). In honey bees, the dendritic sheath may be fused with the wall of the sensillum thereby producing a double-walled hair (Whitehead and Larsen,1976).

Such a sensillum with two lumina was first described by Tinbergen (1939) in the blowfly, *Calliphora erythrocephala*. Later Dethier (1955) and Larsen (1962) described similar sensilla on the labellum of the blowfly, *Phormia regina*. These labellar sensilla in *P.regina* had 3 to 5 dendrites (Larsen, 1962) and were chemosensitive.

On cerci of the Australian sheep blowfly, *Lucilia cuprina*, there are 10 such 'double-channelled' hairs which are presumed to be gustatory (Merritt and Rice,1984). Six of these are innervated by 3 dendrites, the others by 4. In this group of sensilla, the dendrites do reach the apical pore while the second channel does not. It is believed that the second channel is secreted by the cytoplasm of the inner and outer sheath cells. During electrophysiological recording, better signal-to-noise ratios were obtained from the 3-celled than the 4-celled hairs.

Similar apical hairs observed in both larvae and adults of *E.americana* were proposed to be contact chemosensilla that have secondarily evolved a new sensory mode, possibly thermoreception (Sutcliffe and Mitchell, 1980). However, the ultrastructure of the apical hairs in both chrysomelids does not fit exactly the description of hygro/thermorecetors given by Altner *et al*,(1983). Such sensilla are essentially non-porous with an inflexible socket. In cross-sections, the dendrites are closely packed within the dendritic sheath often forming a triad (Loftus,1976) or a diad. Often, a fourth dendrite may be present, the outer segments of which do not reach the base of the peg.

In *L.decemlineata* larvae, Mitchell and Schoonhoven (1974) were able to obtain recordings from the apical hairs with a tip-electrode. However, responses were low frequency, irregular and not correlated with any of the chemical stimuli applied. They nevertheless concluded that the sensilla were chemosensitive. The ultrastructural observations presented here do not refute this.

### MECHANOSENSORY HAIRS

The mechanosensilla present on the galeae are singly innervated. Distally, the dendrite is surrounded by a dendritic sheath (ds) which invaginates at several points often compartmentalizing it (Fig. 3.15). At the base of the hair, the dendrite forms a tubular body (Fig. 3.16, tb). More proximally towards the ciliary region, the dendrites show a typical 9x2+0 ciliary arrangement (Fig. 3.17).

### HOMOLOGIES BETWEEN THE GALEAL SENSILLA OF LARVAE AND ADULTS

In larvae of the Colorado potato beetle, two sensilla termed the 'lateral' and 'medial' sensilla were observed on the galeal tip in addition to the numerous 'setae' which are not chemosensitive (Mitchell and Schoonhoven,1974). SEM and elelctrophysiological studies indicate that the 'lateral' sensillum is chemosensitive. Because of the unique response of this sensillum to L-alanine and GABA, it is evident that this sensillum corresponds to the α-sensillum in adults. The 'medial' sensillum from which no interpretable responses were obtained corresponds to the apical hairs in adults. The difference between larval and adult sensilla is in their number. There are 11 to 15 apical pegs in adults, while there is only 1 in larvae. In addition, larvae have only 1 apical hair ('medial' sensillum) while adults have two.

However, physiological differences between larval and adult sensilla are not seen, and both respond best to specific amino acids. Similarities such as these were observed also in the larval and adult galeal sensilla of the red turnip beetle, *E.americana* (Mitchell *et al*,1979, Sutcliffe and Mitchell,1980).

**Fig. 3.1** : Scanning electron micrograph of ventral view of mouthparts of adult *L.decemlineata*. md,mandibles; ga,galea;lp,labial palpi (Scale:200µm).

**Fig. 3.2** : Scanning electron micrograph of the tip of the galea showing the chemosensitive pegs surrounded by mechanosensory hairs. (Scale:100µm).

**Fig. 3.3** : TEM cross-section of base of an apical peg along the hair shaft showing four chemosensitive dendrites (1-4). ds,dendritic sheath (Scale:1µm).

**Fig. 3.4** : TEM cross-section of base of an apical peg distal to the ciliary region. ds,dendritic sheath; in,inner sheath cell; ss,sensory sinus; tb,tubular body. (Scale:1µm).

**Fig. 3.5** : TEM cross-section of the α-sensillum at outer dendritic segments showing three chemosensitive cells (1-3). ds,dendritic sheath; in,inner sheath cell; ss,sensory sinus;tb,tubular body. (Scale:1µm).

**Fig. 3.6** : TEM cross-section of the α-sensillum along the hair shaft. Note the truncated appearance of dendrites. ds,dendritic sheath. (Scale: 1µm).

**Fig. 3.7** : TEM cross-section of the α-sensillum at the level of ciliary region.in,inner sheath cell; r,cilairy rootlet (Scale: 1µm).

**Fig. 3.8** : TEM cross-section of an apical peg through the proximal region of the dendritic sheath where the inner sheath cell wraps completely around the sheath. in,inner sheath cell; it,intermediate sheath cell;r,ciliary rootlet; ssg,goblet like sinus in the intermediate sheath cell. Scale: 1µm).

**Fig. 3.9** : TEM cross-section of an apical peg at the level of proximal dendritic segments. Scolopale rods (arrows) are seen at the junction of the dendrites and inner sheath cell. d,dendrites;in, inner sheath cell; it,intermediate sheath cell. (Scale: 1µm).

**Fig. 3.10** : TEM cross-section of an apical peg through the inner and intermediate sheath cells and proximal dendritic segments (d) wrapped separately by the inner sheath cell. in,inner sheath cell; it,intermediate sheath cell. Scale:1µm).

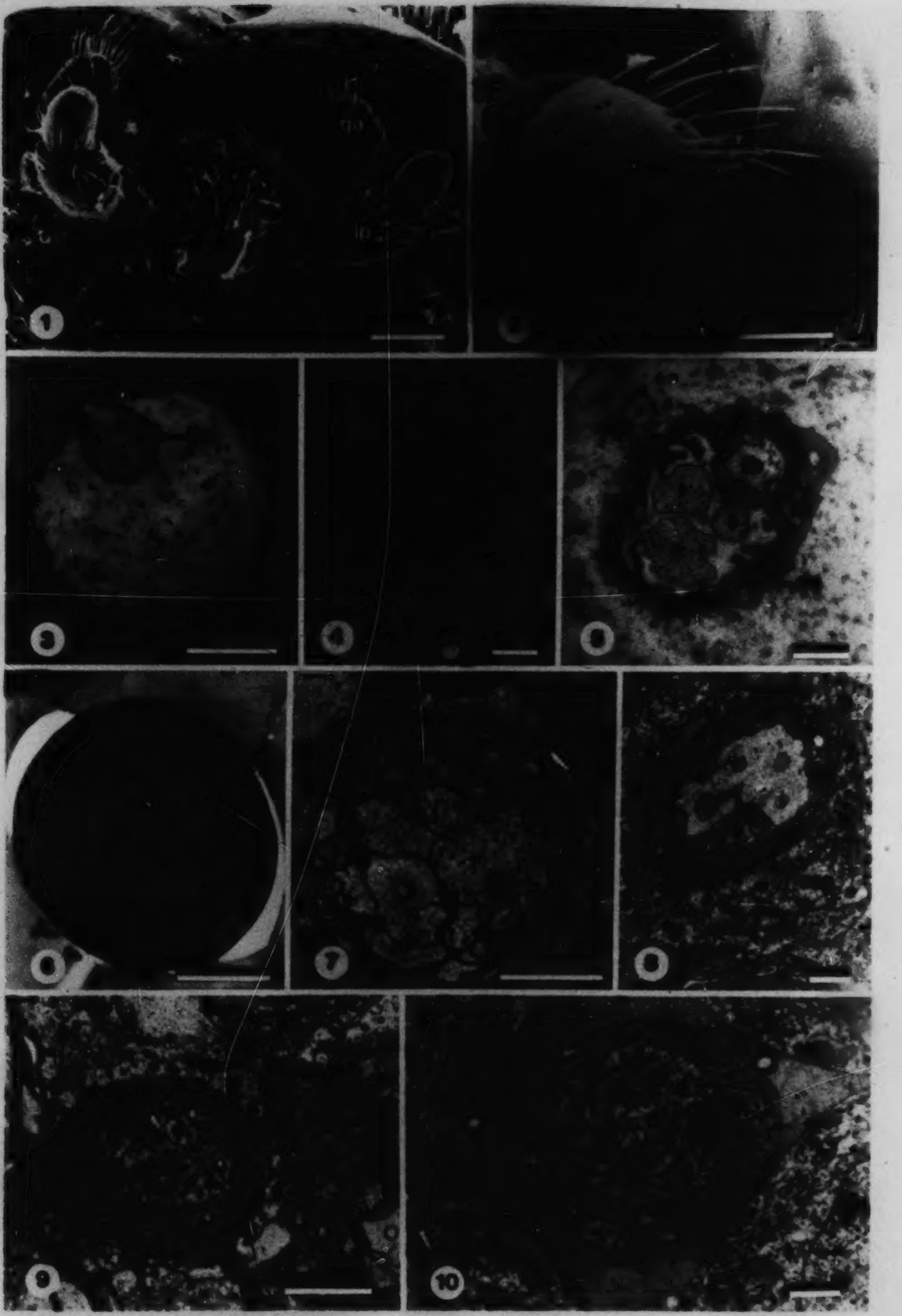
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**Fig. 3.11** : SEM micrograph of the galeal tip showing the two apical hairs (arrows). (Scale: 40µm).

**Fig. 3.12** : SEM micrograph of the tip of an apical hair. Note the finger-like projections (arrows) surrounding the pore. (Scale: 2µm).

**Fig 3.13** : TEM cross-section of an apical hair along the hair shaft. The two dendrites (1,2) lacking a sheath are separated from another chamber, the sensillar lumen (sl). (Scale: 1µm).

**Fig. 3.14** : TEM cross-section through the base of an apical hair.d,dendrites; ds,dendritic sheath;in,inner sheath cell; ss,sensory sinus (Scale: 0.5µm).

**Fig. 3.15** : TEM cross-section of a mechanosensory hair. Note the invaginations of the dendritic sheath. ds,dendritic sheath. (Scale: 1µm).

**Fig. 3.16** : TEM cross-section through the base of a mechanosensory hair. tb,tubular body; ss,sensory sinus. (Scale: 0.5µm).

**Fig. 3.17** : TEM cross-section at the ciliary region of a mechanosensory hair. Note the prominent scolopale rods. in,inner sheath cell; it,intermediate sheath cell;r,ciliary rootlet. (Scale: 1µm)

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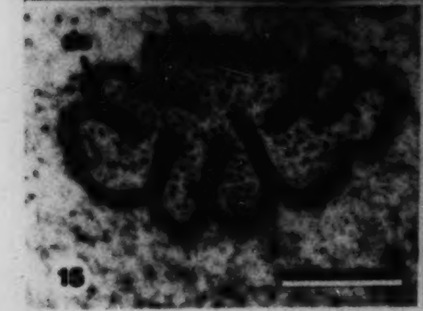
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## REFERENCES

Albert,PJ (1980) Morphology and innervation of mouthpart sensilla in larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can.J.Zool. 58:842-851.

Altner,H, Schaller-Selzer,L, Stetter,H and Wohlrab,I (1983) Poreless sensilla with inflexible sockets. A comparative study of a fundamental type of insect sensilla probably comprising thermo- and hygroreceptors. Cell Tissue Res. 234:279-307.

Bernays,EA, Cook,A and Padham,DE (1976) A club-shaped hair found on the first instar nymphs of *Schistocerca gregaria*. Physiol. Entomol. 1:3-13.

Blaney,WM and Chapman,RF (1969) The fine structure of the terminal sensilla on the maxillary palps of *Schistocerca gregaria* (Forskal) (Orthoptera, Acrididae). Z.Zellforsch.Mikro.Anat. 99:74-97.

Blaney,WM and Chapman,RF (1970) The functions of the maxillary palps of Acrididae (Orthoptera). Ent. exp. appl. 13: 363-376.

Blaney,WM, Chapman,RF and Cook,AG (1971) The structure of the terminal sensilla on the maxillary palps of *Locusta gregaria* (L.) and changes associated with moulting. Z.Zellforsch.Mikro.Anat. 121:48-68.

De Boer,G (1985) The roles of phytochemicals, chemosensory organs and feeding experience in food plant selection by the larvae of the tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingidae). Ph.D. Dissertation, University of Maryland, Baltimore.

De Boer,G, Dethier,VG and Schoonhoven,LM (1977) Chemoreceptors in the preoral cavity of the tobacco hornworm,*Manduca sexta* and their possible function in feeding behavior. Ent.exp.appl. 21:287-298.

Dethier,VG (1955) The physiology and histology of the contact chemoreceptors of the blowfly. Quart.Rev.Biol. 30:348-371.

Hsiao,T (1974) Chemical influence on feeding behavior of *Leptinotarsa decemlineata*. In: Experimental Analysis of Behavior. L.Barton Browne (Ed.)Springer Verlag, New York.:237-248.

Ishikawa,S (1965) Maxillary chemoreceptors in the silkworm. In : Olfaction and Taste II. T.Hayashi (ed.). Pergamon

Pres,Oxford.

Kaissling,KE (1971) Insect olfaction. In : Handbook of sensory Physiology. Springer Verlag, Berlin. : 351-431.

Klein,U (1981) Sensilla of the cricket palp. Fine structure and spatial organization. Cell Tissue Res. 219:229-252.

Klein,U and Müller,B (1978) Functional morphology of the palp sensilla related to food recognition in *Gryllus bimaculatus* (Saltatoria:Gryllidae). Ent.exp.appl. 24:491-495.

Larsen,JR (1962) The fine structure of the labellar chemosensory hairs of the blowfly, *Phormia regina* Meigen. J.Insect Physiol.8:683-691.

Loftus,R (1976) Temperature dependent dry receptor on antenna of *Periplaneta*. Tonic response. J.Comp.Physiol. 111: 153-170.

Ma,WC (1972) Dynamics of feeding responses in *Pieris brassicae* Linn. as a function of chemosensory input: a behavioral, ultrastructural and electrophysiological study. Mededelingen van de Landbouwhoogeschool te Wageningen, pp 72-11.

McIver,SB (1982) Sensilla of mosquitoes (Diptera:Culicidae). J.Med.Entomol. 19:489-535.

Merritt,DJ and Rice,MJ (1984) Innervation of the cercal sensilla on the ovipositor of the Australian sheep blowfly (*Lucilia cuprina*). Physiol.Entomol. 9: 39-47.

Mitchell,BK (1974) Behavioral and electrophysiological investigations on the responses of ~~larvae of the~~ Colorado potato beetle (*Leptinotarsa decemlineata*) to amino acids. Ent.exp.appl. 17: 255-264.

Mitchell,BK (1985) Specificity of an amino acid sensitive cell in the adult Colorado beetle, *Leptinotarsa decemlineata*. Physiol.Entomol.10:421-429.

Mitchell,BK and Gregory,P (1979) Physiology of the maxillary sugar sensitive cell in the red turnip beetle, *Entomoscelis americana* Brown.J.Comp.Physiol.(A) 132: 167-178.

Mitchell,BK and Harrison,GD (1984) Characterization of galeal chemosensilla in the adult Colorado beetle,*Leptinotarsa decemlineata*. Physiol.Entomol.9:49-56.

Mitchell,BK and Harrison,GD (1985) Effects of Solanum glycoalkaloids on chemosensilla in the Colorado potato beetle : a mechanism of feeding deterrence? J.Chem.Ecol. 11: 73-83.

Mitchell,BK and Schoonhoven,LM (1974) Taste receptors in Colorado beetle larva. J.Insect Physiol. 20:255-264.

Mitchell,BK, Whitehead,AT and Backus,E (1979) Ultrastructure of the lateral and medial galeal sensilla of the larva of the red turnip beetle, *Entomoscelis americana* Brown (Coleoptera:Chrysomelidae). Int.J.Insect Morph. & Embryol. 8:289-295.

Rice MJ, Galun,R and Finlayson,LH (1973) Mechanotransduction in insect neurones. Nature: New Biology 241: 286-288.

Schoonhoven, LM (1972) Plant recognition by lepidopterous larvae. Insect/Plant Relationships:Symposium of the Royal Entomological Society, London. HF van Emden (ed.):87-99.

Schoonhoven,LM and Dethier,VG (1966) Sensory aspects of host plant discrimination by lepidopterous larvae. Arc.Neer. Zool. 16: 497-530.

Steinbrecht,RA (1980) Cryofixation without cryoprotectants. Freeze substitution and freeze etching of an insect olfactory receptor. Tissue and Cell. 12: 73-100.

Sutcliffe,JF and Mitchell,BK (1980) Structure of galeal sensory complex in adults of the red turnip beetle, *Entomoscelis americana* Brown (Coleoptera, Chrysomelidae). Zoomorphologie. 96: 63-76.

Tinbergen,L (1939) Uber den Bau der Geschmacksorgane auf dem Proboscislippen und den Beinen von *Calliphora erythrocephala* Meig. Arch. Neer. Zool 4:82-92.

Whitehead,AT and Larsen,JR (1976) Ultrastructure of the contact chemoreceptors of *Apis mellifera* L. (Hymenoptera:Apidae). Int.J.Insect Morph. & Embryol. 5:301-315.

Wieckzorek,H (1976) The glycoside receptor of the larvae of *Mamestra brassicae* L. (Lepidoptera:Noctuidae). J.Comp. Physiol. (A). 106:153-176.

## IV. Chemical composition and ultrastructure of epicuticular waxes from leaves of *Solanum tuberosum*

### INTRODUCTION

Aerial surfaces of all plants are covered by a thin impervious layer of epicuticular wax occuring mainly on leaves and fruits. A variety of functions have been attributed to this lipid layer emphasizing its involvement in both physical and physiological processes occuring within the primary surface tissue (see reviews by Kolattukudy and Walton,1972; Kolattukudy,1972 and Tulloch,1976).

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), feeds on potato and some other solanaceous species. Adults have a characteristic pattern of feeding behavior in which they tap the leaf surface extensively with their maxillary palpi before taking a bite (personal observations). The present study was undertaken to partially determine the chemical composition of leaf surface waxes of potato in order to identify components which may be involved in host recognition by the adult beetle. In addition, leaf surface waxes from five other solanaceous species, which have been used in behavioral studies, viz., *Solanum elaegnifolium, Lycopersicon esculentum, Nicotiana tabacum, Datura stromonium* and *Solanum nigrum* were analysed by thin layer chromatography (TLC) to identify differences in their chemical composition.

## REVIEW OF LITERATURE

The origins and structure of plant epicuticular waxes have been the subject of increasing interest over the past one hundred years. As early as 1871, De Bary distinguished four main types of wax coating : needles, rods, granular layers and films. In recent years, study of plant surface waxes has intensified as indicated by the increasing number of publications on the subject. This resurgence of interest can be attributed to improvements in methods for investigating their chemical and physical properties and our increasing awareness of the important role of the surface in the normal functioning of the plant. Improved resolving power of transmission electron microscopes and increased depth-of-focus provided by scanning electron microscopes have facilitated ultrastructural study while improved chemical analysis techniques like gas-liquid chromatography (GLC), nuclear magnetic resonance (NMR), infra-red spectroscopy (IR) and mass spectroscopy (MS) have helped to identify even trace amounts of chemical constituents. Such detailed studies have demonstrated that the fine structure of leaf surface waxes is a sufficiently stable characteristic of a plant species to allow its use as a taxonomic criterion.

### STRUCTURE OF LEAF CUTICLE

The cuticle in plants is a continuous, non-cellular, multilayered membrane which lies over the epidermal cells and is almost entirely lipoidal

(Kolattukudy,1975). Epicuticular wax occurs as a layer which rests upon cutin. Cutin, the substance responsible for the structural integrity of the plant cuticle, is composed of polymers of cross-esterified fatty acids (Fig. 4.1). These form the outermost layer or the cuticle proper, and appear to be deposited in the form of ultramicroscopic lamellae with electron dense bands of uniform thickness interspersed with electron-dense bands of variable thickness (Jeffree *et al*, 1976). The electron transparent bands are probably waxy in nature and the electron dense bands form the cutin. Cutin is also found, along with pectin, in the underlying layer which forms the bulk of the cuticle (Holloway *et al*,1977). Beneath the lamellate layer is an inner region consisting of an apparently structureless matrix composed of pectin which fastens the cuticle to the epidermal cell wall.

## CHEMICAL CONSTITUENTS OF WAXES

The lipids associated with epicuticular wax comprise a complex mixture of long chain and cyclic compounds. Hydrocarbons are among the most common and abundant components of epicuticular lipids. *n*-alkane (straight chain) hydrocarbons constitute the principal hydrocarbon fraction, ranging in length from $C_{21}$ to $C_{37}$ carbon atoms with odd-numbered chains predominating. Small percentages of branched alkanes also occur, particularly in tobacco, where they form up to 50% of the hydrocarbons (Tulloch,1976). Branching types include iso (2-methyl) and anteiso (3-methyl) compounds; dimethyl alkanes and internally branched alkanes are rare as are unsaturated (alkene) hydrocarbons (Tulloch,1976).

Oxygenated derivatives of alkanes comprise a second important group of cuticular lipids. These include wax esters, free fatty acids, alcohols, ketones and aldehydes. Wax esters are a common constituent in most plants. They usually consist of free fatty acids with a carbon chain of $C_{20}$ to $C_{24}$ esterified to alcohols having an even number of carbon atoms in the range of $C_{12}$ to $C_{32}$ (Kolattukudy,1975). Free fatty acids and free alcohols also occur but are rarely major components. The alcohols are usually straight chain; even numbered compounds similar in length to the esterified alcohols whereas the fatty acids may be saturated, unsaturated and even branched, and often have chain lengths that exceed those of esterified fatty acids. A variety of ketones and aldehydes appear to be present as major components (Hadley,1981).

Cyclic compounds represent a third category and include triterpenes, flavones, sterols and aromatic hydrocarbons.

## RELATIONSHIP BETWEEN WAX MORPHOLOGY AND CHEMISTRY

During recent years it has become increasingly evident that the form and spatial distribution of crystalline deposits are determined principally by the composition of the wax exudates. From observations on the leaf and leaf sheath waxes of cereals, Lundquist et al (1968) proposed that primary alcohols crystallise as plates and ß-deketones as thin tubes. von Wettstein-Knowles (1974) proposed that ketones are determinants of the 'loofah-like' tubes found on leaves of *Brassica* spp. Epicuticular waxes which produce short stubby tubes always contain substantial amounts of nonacosane-10-ol (Jeffree *et al*,1976). These workers further demonstrated that following fractionation, nonacosane-10-ol recrystallised in a form which closely resembled that found on the intact plant. Although aldehydes rarely occur as a major wax component, their presence in epicuticular wax mixtures correlates closely with production of rod structures (Baker,1982).

## TRANSPORT OF WAXES

Although considerable progress has been made in determining of factors controlling form and composition of epicuticular deposits, little information is available to indicate the routes and mechanisms by which wax constituents or their precursors are transported. The physical and chemical properties of cuticular lipids suggest that they are formed very near the cuticle. The epidermal cells of these plants contain all the enzymes necessary to synthesize fatty acids and convert them to cutin. For the wax to reach the surface, it has to pass through a mixed layer of cutin and wax. As the wax migrates, some of it impregnates the cutin layers of the cuticle.

Various theories have been put forward to explain the deposition of wax on the epicuticle. Hall and Davidson (1962) produced evidence for the existence of cuticular pores and claimed that the structural form of the epicuticular deposits was determined by the number of pores associated with individual wax particles. However, detailed investigations by other workers have failed to confirm the presence of pores or microchannels in certain plant cuticles at any stage of development (Mueller *et al*,1954; Juniper, 1960; Crisp,1966; Leigh and Matthews,1963; Jarvis and Wardrop,1974).

Mueller *et al* (1954) put forward the liquid extrusion theory which favoured the view that wax is extracted under pressure through the cuticle in the form of a paste. A disadvantage of this theory is that since wax constituents have relatively high melting points, they would have to be retained in a super-cooled state prior to extrusion (Baker,1982).

An alternative mechanism, the polymerization theory, proposed by Hallem (1970) envisaged the liberation of wax precursors from Golgi body vesicles followed by diffusion through the cuticular lamellae and polymerization into characteristic wax formations. However, with the exception of aldehydes and estolides, polymeric constituents have not been identified in plant waxes.

During recent years, considerable support has been given to the initial suggestion of Wiesner (1871) who proposed that wax precursors are carried through the cuticle in a volatile solvent and crystallize at the plant surface in a variety of crystalline forms. Baker (1974) gave further support for the involvement of a volatile solvent substance and suggested that the prevailing environmental conditions controlled evaporation rates and thereby determined the ultimate form of the crystalline deposit. Direct evidence in favor of a crystalline mechanism was obtained from experiments with a model system (Jeffree *et al*,1975) which showed that extracted wax could crystallize into wax crystals with shapes and dimensions similar to those on the intact plant surface. They also showed that when wax was fractionated into its different components, and the fraction recrystallized sequentially, the resulting crystals more closely resembled those on the intact leaf surface than when the whole wax was recrystallized.

Thus, it may be that after wax synthesis, the epidermal cells and cuticle play no further role in the development of the ultrastructure of plant surface waxes. The fact that extracted wax can be recrystallized in a form identical to that on a plant surface is evidence against any chemical modification of the wax at the plant surface after excretion. The crystallization theory assumes that the epidermal cells contain enough volatile solvents to carry the wax to the surface, but it is not known if this is the case. The carrier solvents would have to be organic solvents because the wax is insoluble in an aqueous solution. Some possible solvents that have been suggested are short-chain aldehydes, ketones, alcohols and 1-hexane (Tulloch,1976).

## FUNCTION OF LEAF SURFACE WAXES

Leaf epicuticular waxes occur as an inert layer between the plants and the environment. This layer effectively reduces water loss due to transpiration (Grncarevic and Radler,1967; Denna,1970), contributes to the control of gaseous exchange (Jeffree *et al*, 1971), prevents loss of essential nutrients and organic solvents due to leaching (Tukey,1971) and influences the retention and redistribution of foliar applied chemicals (Holloway,1970). It also provides a microhabitat for a variety of parasitic and saprophytic organisms (Pady,1971) and acts as a barrier to fungal pathogens (Martin and Juniper,1970).

# MATERIALS AND METHODS

## PLANT MATERIAL

Potato plants (variety : Norland) were grown in the field under normal agronomic conditions without the application of pesticides. Other plants viz., *Nicotiana tabacum, Solanum elaeagnifolium, Lycopersicon esculentum, Datura stromonium* and *Solanum nigrum* were grown in 10 inch pots in the greenhouse. Leaves were collected from 10 week old plants, put in polythene bags and brought to the laboratory for extraction of leaf surface waxes.

## SCANNING ELECTRON MICROSCOPY

Fresh leaves, and those from which wax had been removed by dipping in solvent, were fixed in osmium vapour by placing leaves overnight in petri dishes containing 1% osmium tetroxide in phosphate buffer (pH 7.0). The leaves were air dried for 3 days, sputter coated with gold in a Nano-Semprep 2 and observed under a Cambridge SEM 250 scanning electron microscope operating at 20Kv.

## EXTRACTION OF WAX

Leaf surface waxes were extracted by dipping leaves in chloroform for 10 seconds. Care was taken so that damaged leaves or any cut portion was not immersed in chloroform. Anhydrous $MgSO_4$ was added to dry the crude extract which was then filtered. The extract was evaporated in a flash point evaporator (Buchi, Rotavapor R) and the crude wax collected and weighed.

### FRACTIONATION

Detailed analyses were carried out only with potato leaf surface waxes. The procedure for separating wax into its constituents was done following the method described by Kollatukudy (1970). The crude wax (1.3745 g) was redissolved in a small volume of chloroform. A silica gel column (2x40 cm) was made using silicic acid (Biosil A,100-200 mesh) dissolved in heptane. The redissolved wax was loaded on top of the column. The following solvents were then passed through the column :

| | |
|---|---|
| n-hexane (redistilled) | 200 ml |
| Benzene | 400 ml |
| Chloroform | 480 ml |
| Methanol | 520 ml |

Twenty-five ml aliquots were collected using a fraction collector and concentrated to about 2-3 ml. TLC was done using glass microscope slides. Benzene was used as the developing solvent and spots were obtained after heating slides sprayed with 50% sulphuric acid. Aliquots with the same Rf value were pooled together. Semipreparative TLC was done using silica gel coated plates (containing an indicator so that spots could be visualized under UV light). Bands were eluted by scraping off the silica gel, dissolving them in chloroform, filtering and then spotting them on TLC plates. Using solvent systems like benzene:chloroform (7:3) and chloroform:hexane (9:1), TLC was done till a single spot was obtained. Such samples were then analysed by FT-IR, GLC and MS.

## FOURIER TRANSFORMATION INFRA-RED SPECTROSCOPY (FT-IR)

FT-IR spectra were obtained with a Nicolet MX-1 spectrophotometer using a KCl cell.

## GAS-LIQUID CHROMATOGRAPHY

The crude wax extracts from potato leaves and those from *S.elaegnifolium*, *S.nigrum*, *N.tabacum*, *D.stromonium* and *L.esculentum* were redissolved in chloroform and analysed by gas-liquid chromatography (GLC). The individual components of leaf surface waxes of potato, as obtained through semi-preparative TLC, were also analysed by GLC following the method described by Flore and Bukovac (1978) and Tulloch (1983). One µl (10 µg/ml) of each sample was chromatographed using a Hewlett Packard Model 5830H gas chromatograph. Flow rate of the helium carrier gas was 50 ml/min. The column used was 3mm x 1.2m stainless steel containing 1% Dexil 300. The oven temperature was programmed to increase from 120$^0$C to 330$^0$C at a rate of 30 $^0$C/min. The injection port and the hydrogen flame ionization detector temperatures were 325$^0$C and 330$^0$C, respectively. Chain lengths of *n*-alkanes were determined by comparisions of peak retention times with retention times of known hydrocarbon analytical standards (Analabs-New England Nuclear, Polychem

Corp). Identification of other compounds were made specifically by analyses of MS data as has been done to elucidate the structure of a number of wax components by a number of workers (Kollatukudy,1970 and Stoinova-Ivanova and Mladeva,1971) and by comparing the GLC data by Bukovac *et al*,1979; Flore and Bukovac,1978 and Knowles and Flore,1983.

## RESULTS AND DISCUSSION

Leaf surfaces of potato are covered by a thick, continuous layer of amorphous wax supporting ribbons of crystalline wax (Fig. 4.2). Ribbons are the most common form of crystalline wax having been reported from a number of plants including *Fragaria* and *Rosa* sp. (Baker,1982). A ten second extraction of the leaves in chloroform was sufficient to remove epicuticular waxes (Fig. 4.3). Longer durations resulted in extraction of more than just the epicuticular waxes as indicated by a greenish tinge in the extract.

Wax yields from the six solanaceous plant species show sparse wax deposits (Table 4.1). Amounts of epicuticular wax extracted varied from 2.1 µg $cm^{-2}$ in *S.nigrum* to 10.6 µg $cm^{-2}$ in *S.elaegnifolium*. Leaves of many herbaceous plants such as *Lactuca sativa, Spinacea oleracea* and *Beta vulgaris* have a thin wax layer with only 5-10 µg $cm^{-2}$ (Baker,1982) in contrast to leaves of *Allium porrum, A.cepa* and *Brassica sp.* which have a heavier deposit of 30-60 µg $cm^{-2}$ (Baker,1982). Thicker deposits ranging from 60-300 µg $cm^{-2}$ have been reported from leaves of *Ceratonia siliqua, Pistacia lentiscus* and *Olea europaea* grown under arid conditions (Baker and Procopiou,1980).

The composition of epicuticular waxes from potato and those of other solanaceous species were analysed by TLC and compared with those of cabbage (Table 4.2). The waxes were separated into eight bands representing 8 different classes of constituents which were identified with the help of FT-IR spectra and from comparison of data presented by Bukovac *et al*,1979; Flore and Bukovac,1978; and Knowles and Flore,1983. In order of elution on TLC plates, the classes of constituents included *n*-alkanes, wax esters, ketones, aldehydes, *s*-alcohols, ketols, *p*-alcohols and acids. There were no marked differences in the presence of major classes of compounds except for the absence of ketols in the solanaceous species investigated. The percent composition of the major classes of compounds in *S.tuberosum* is listed in Table 4.3. *n*-alkanes form the principal component with about 39% of total wax. Wax esters, aldehydes and *s*-alcohols form the other major components.

Comparision of GLC profiles of leaf surface waxes from plants used in this study were made with data presented by Bukovac *et al*,1979; Flore and Bukovac,1978 and Knowles and Flore,1983. There are major differences in the amounts of the various components (Figs. 4.4,4.5). While all the plants examined had prominent alkane peaks (9,11,12,14), there is considerable variation amongst the other components. Peaks 1 through 8 include the aldehydes, *p*-alcohols, ketones and *s*-alcohols. *S.elaeagnifolium* is characteristic in having a higher proportion of these compounds as compared to the other plants. It also appears very likely that *S.elaeagnifolium* has prominent lower chain *n*-alkanes like $C_{23}$, $C_{25}$ since the $C_{31}$ peak (14) is appreciably smaller in area as compared to *S.tuberosum* and *L.esculentum*. Peak 12 in *S.tuberosum* is primarily due to $C_{29}$

*n*-alkane and also due to $C_{28}$ aldehyde. Esters are often a common constituent of the leaf surface and they appear to be present in all of the plants investigated (15,16,17,18).

### HYDROCARBONS

The hydrocarbon fraction consisted of *n*-alkanes ranging from $nC_{25}$-$nC_{33}$ with only odd carbon numbered homologues. FT-IR spectra of this fraction showed absorbance at 1460 $cm^{-1}$, 1470 $cm^{-1}$ and at 2920-2841 $cm^{-1}$ indicating the presence of hydrocarbons. No branched chain alkanes were detected. GLC analyses revealed the presence of $nC_{25}$-$nC_{33}$ straight chain alkanes when compared with the retention times of n-alkane standards (Fig. 4.6). $n$-$C_{31}$ was the predominant compound with $C_{25}$, $C_{27}$, $C_{29}$ and $C_{33}$ forming the other major components. MS data confirmed the presence of $C_{25}$-$C_{33}$ *n*-alkanes with peaks corresponding to $n$-$C_{25}$ ($M^+$ m/z 352), $n$-$C_{27}$ ($M^+$ m/z 380), $n$-$C_{29}$ ($M^+$ m/z 408), $n$-$C_{31}$ ($M^+$ m/z 436) and $n$-$C_{33}$ ($M^+$ m/z 464). A major component of surface waxes of potato is *n*-alkanes (39%) of which $C_{31}$ is predominant. *n*-alkanes are often the major component of leaf surface waxes. In *Solandra grandiflora*, they account for 92% of the total wax with the highest molecular concentration per unit area (Herbins and Robins,1968). Of the *n*-alkanes, $C_{31}$ is the most common reported so far, being almost the only one in candelilla (Tulloch,1976) and peas (Kolattukudy, 1970). In tobacco, it is the predominant *n*-alkane along with odd chain *iso* (2-methyl) and even chain *anteiso* (3-methyl) compounds (Carruthers and Johnstone, 1959).

### PRIMARY ALCOHOLS

The primary alcohol fraction consisted entirely of even chain length homologues ($C_{18}$-$C_{34}$). FT-IR spectra of this fraction indicate absorbance at 1260-1000cm$^{-1}$ and at 1420-1330cm$^{-1}$. Mass spectra of this fraction gave diagnostic ions ($M^+$ -18) corresponding to $C_{18}$(m/z 252), $C_{20}$ (m/z 280), $C_{22}$ (m/z 308), $C_{24}$ (m/z 336), $C_{26}$ (m/z 364), $C_{28}$ (m/z 392),$C_{30}$ (m/z 420), $C_{32}$ (m/z 448) and $C_{34}$ (m/z 470).

### WAX ESTERS

FT-IR spectra of the unhydrolysed fraction showed absorbance at 1735 cm$^{-1}$. Fragments corresponding to $n$-$C_{42}$ ($M^+$ m/z 538) and $n$-$C_{44}$ (M+ m/z 566) wax esters could be identified. Peaks corresponding with R-OH fragments were observed at $n$-$C_{22}$ ($M^+$ m/z 326), $n$-$C_{24}$ ($M^+$ m/z 354), $n$-$C_{26}$ ($M^+$ m/z 382) while those for $RCOOH_2$ were obtained for $n$-$C_{18}$ ($M^+$ m/z 285), $n$-$C_{20}$ ($M^+$ m/z 313).

### KETONES

The ketone fraction separated by semipreparative TLC gave an FT-IR spectrum with absorbance at 1718 cm$^{-1}$. Fragments corresponding to $n$-$C_{25}$ ($M^+$-15 m/z 351) and $n$-$C_{23}$($M^+$-15 m/z 323) were obtained.

### SECONDARY ALCOHOLS

FT-IR spectra showed absorption at 1195 cm$^{-1}$ confirming the presence of $s$-alcohols. MS identification revealed fragments of $n$-$C_{23}$ ($M^+$-18 m/z 322) and $n$-$C_{25}$ ($M^+$ -18 m/z 350).

## ALDEHYDES

Absorbance at 1724 $cm^{-1}$ of the aldehyde fraction confirmed the presence of aldehydes. Peaks corresponding with n-C22 (M+ -28 m/z 324), n-$C_{24}$ ($M^+$-28 m/z 352), n-$C_{26}$ ($M^+$-28 m/z 380) and n-$C_{28}$ ($M^+$-28 m/z 408) were identified in the MS data.

## ACIDS

The acid fraction consisted of even chain-length homologues ranging from $C_{18}$-$C_{34}$. FT-IR spectra revealed absorbance at 1760 $cm^{-1}$. Fragments corrsponding to $M^+$-COOH were identified for n-$C_{18}$ (m/z 239), n-$C_{20}$ (m/z 267), n-$C_{22}$ (m/z 295), n-$C_{24}$ (m/z 323), n-$C_{26}$ (m/z 351), n-$C_{28}$ (m/z 379), n-$C_{30}$ (m/z 407), n-$C_{32}$(m/z 435), n-$C_{34}$(m/z 463) and n-$C_{36}$ (m/z 491).

In the present study, it appears that leaf surface waxes of potato consist of a mixture of compounds commonly seen in waxes of other plant species. Although wax morphology is under genetic control (Bianchi and Marchesi, 1960; Lundqvist & von Wettstein,1968), the configuration, size and distribution of the crystalline waxes can be significantly modified by prevailing environmental conditions. In addition, wax morphology is closely associated with chemical composition.

Current concepts suggest that wax components are biosynthetically related. Kolattukudy (1966) suggested an elongation-decarboxylation hypothesis for the biosynthesis of hydrocarbons in plants. According to this hypothesis, the usual end product of fatty acid synthetase, palmitic acid ($C_{16}$) is the substrate for an elongation-decarboxylation system to which acetate ($C_2$) units are added until the chain length reaches $C_{30}$ or $C_{32}$. Decarboxylation of this acid results in formation of the major alkane. This is then further oxidised to form the major secondary alcohols and ketones. In *Brassica oleracea*, the principal *n*-alkane is $C_{31}$ and the corresponding ketones and *s*-alcohols are also found (Kolattukudy,1966). However, in peas, with $C_{31}$ as the major alkane, only the corresponding alcohol is seen (Kolattukudy, 1970). It has been shown however, that where there are mixtures of alkanes, the corresponding mixtures of s-alcohols are also present (Wollrab,1969).

In the present investigation on leaf surface waxes of potato, the *s*-alcohol and ketones were observed to have identical chain lengths of $C_{23}$ and $C_{25}$. The *n*-alkanes ranged from $C_{23}$ to $C_{33}$ in chain length. In addition, since the aldehydes are intermediates in the conversion of acids to alcohols (Kolattukudy,1970), the aldehydes would be expected to resemble the alcohols. In leaf surface waxes of potato, the aldehydes ($C_{22}$-$C_{28}$) and *p*-alcohols ($C_{18}$-$C_{34}$) are similar and are of sufficient length to have been derived by the reduction of fatty acids via the fatty acid elongation pathway. The *p*-alcohol chain length is similar to that of the alcohol moeity in wax esters thereby suggesting a common origin in which they participate in the sterification process. Variation in the fatty acid moeity of the ester indicates an origin from different sources - probably enzyme mediated as has been suggested in a number of plants (cf. Kolattukudy and Walton,1972).

Table 4.1 : Amounts of leaf epicuticular waxes from six solanaceous species.

| Species | Amount ($\mu g\ cm^{-2}$) |
|---|---|
| *S.tuberosum* | 5.4 |
| *S.elaegnifolium* | 10.6 |
| *L.esculentum* | 7.3 |
| *S.nigrum* | 2.1 |
| *N.tabacum* | 8.5 |
| *D.stromonium* | 3.9 |

**Table 4.2** : Rf values of epicuticular wax components of six solanaceous plants compared with those of cabbage

| Major components | *B.oleracea* | *S.tuberosum* | *S.eleagnifolium* | *N.tabacum* | *L. esculentum* | *S.nigrum* | *D.stromonium* |
|---|---|---|---|---|---|---|---|
| Alkane | 0.98 | 0.97 | 0.97 | 0.97 | 0.97 | 0.97 | 0.96 |
| Ester | 0.63 | 0.72 | 0.70 | 0.77 | 0.74 | 0.74 | 0.74 |
| Ketone | 0.54 | 0.55 | 0.55 | 0.55 | 0.70 | 0.63 | 0.54 |
| Aldehyde | 0.34 | 0.32 | 0.31 | 0.33 | 0.36 | 0.30 | 0.30 |
| *s*-alcohol | 0.28 | 0.27 | 0.25 | 0.25 | 0.26 | 0.27 | 0.27 |
| Ketol | 0.17 | - - | - - | - - | - - | - - | - - |
| *p*-alcohol | 0.11 | 0.11 | 0.13 | 0.11 | 0.12 | 0.11 | 0.11 |
| Fatty acids | 0.05 | 0.05 | 0.06 | 0.04 | 0.06 | 0.04 | 0.05 |

Table 4.3 : Percent composition of major classes of compounds from leaf surface waxes of *S.tuberosum*

| Component Class | Per cent |
|---|---|
| *n*-alkanes | 38.96 |
| Ketones | 5.91 |
| Esters | 19.63 |
| *s*-alcohols | 14.24 |
| Aldehydes | 8.55 |
| *p*-alcohols | 10.23 |
| Acids | 2.15 |

**Fig. 4.1** : Diagramatic representation of the leaf cuticle. EW, epicuticular wax; LR, lamellate region; O, outer layer; I, Inner layer; CW, cell wall.

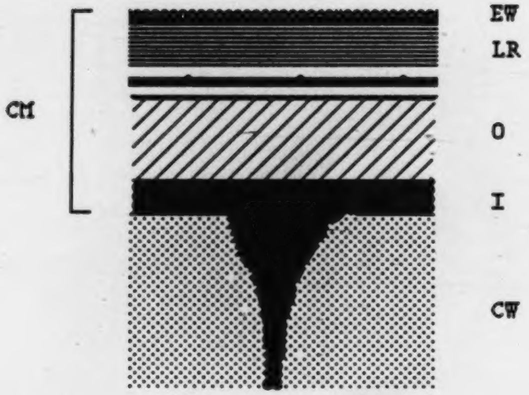
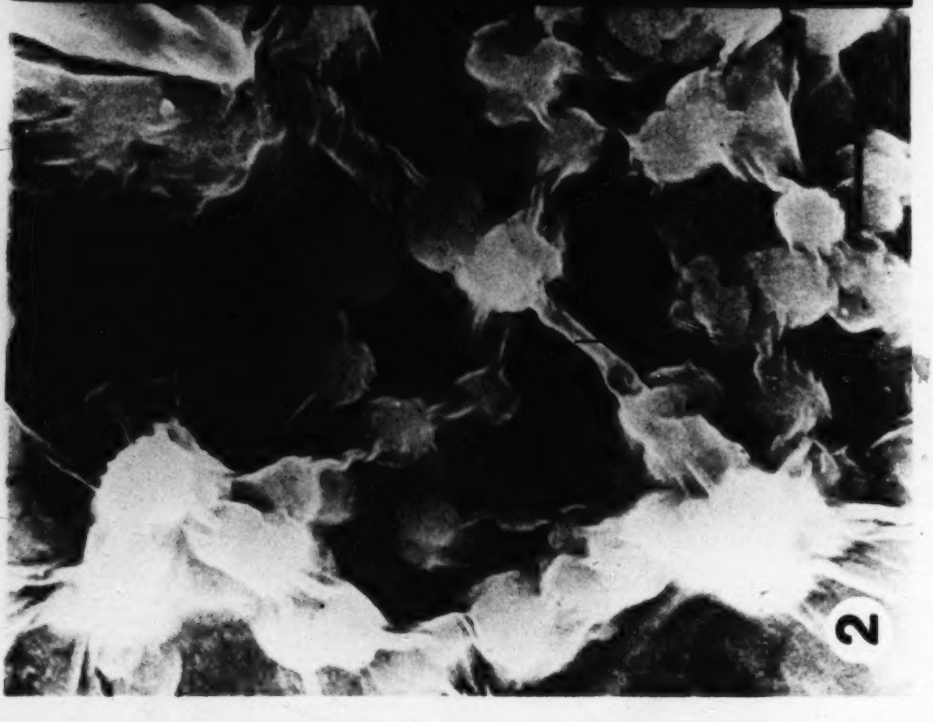

CM
EW
LR
O
I
CW

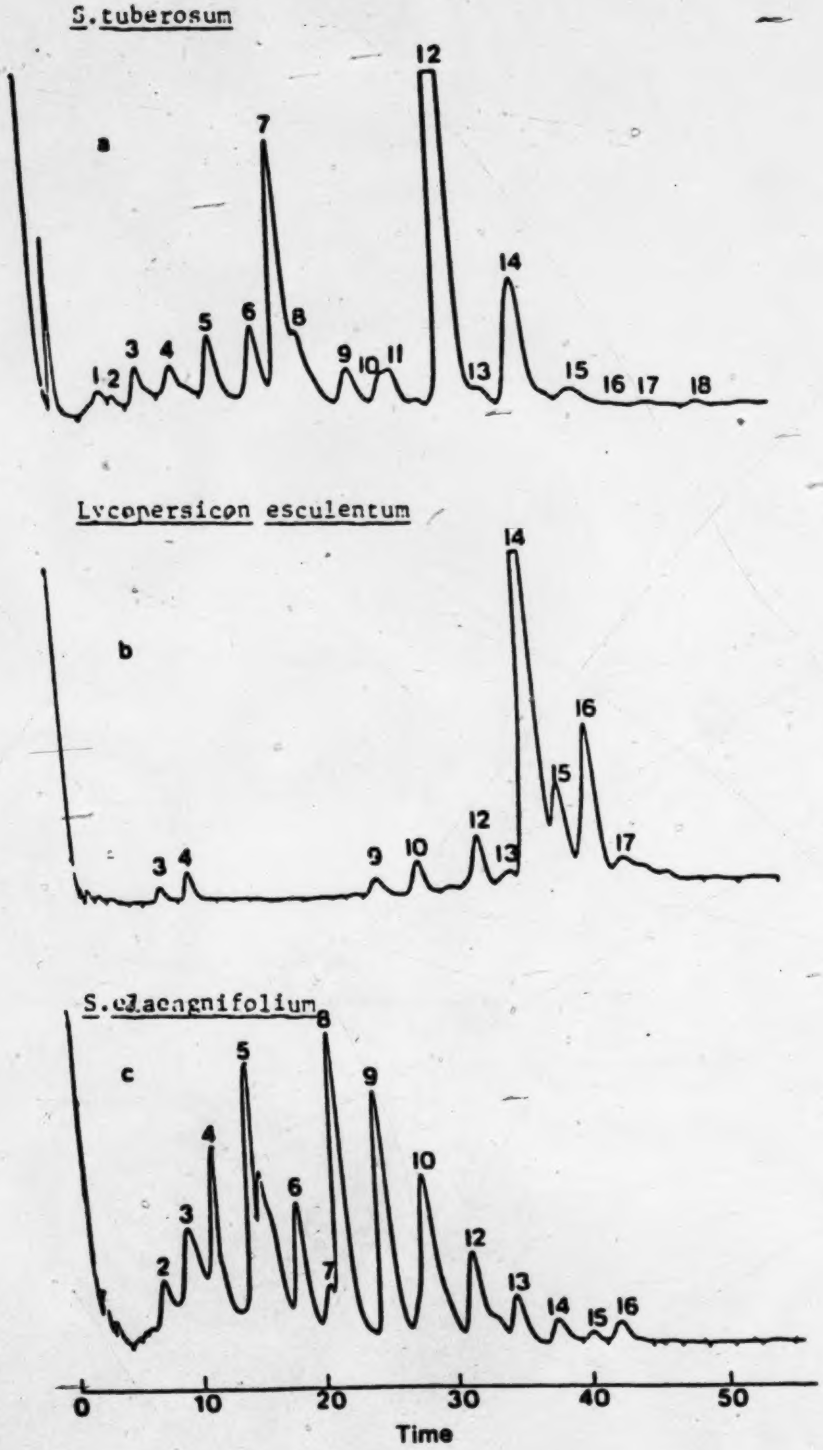
**Fig. 4.2** : SEM micrograph of the leaf cuticle showing distribution of crystalline wax in the form of ribbons (Scale=10µm).

**Fig. 4.3** : SEM micrograph of the leaf surface of potato after treating with chloroform for 10 seconds (Scale=40µm).


2

3

**Fig. 4.4** : GLC profile of epicuticular waxes isolated from leaves of a) *S.tuberosum;*b) *L.esculentum* and c) *S.elaeagnifolium*. 1-8: *p*-alcohols, aldehydes, ketones and *s*-alcohols;9,11,12,14: alkanes; 15-18 : esters

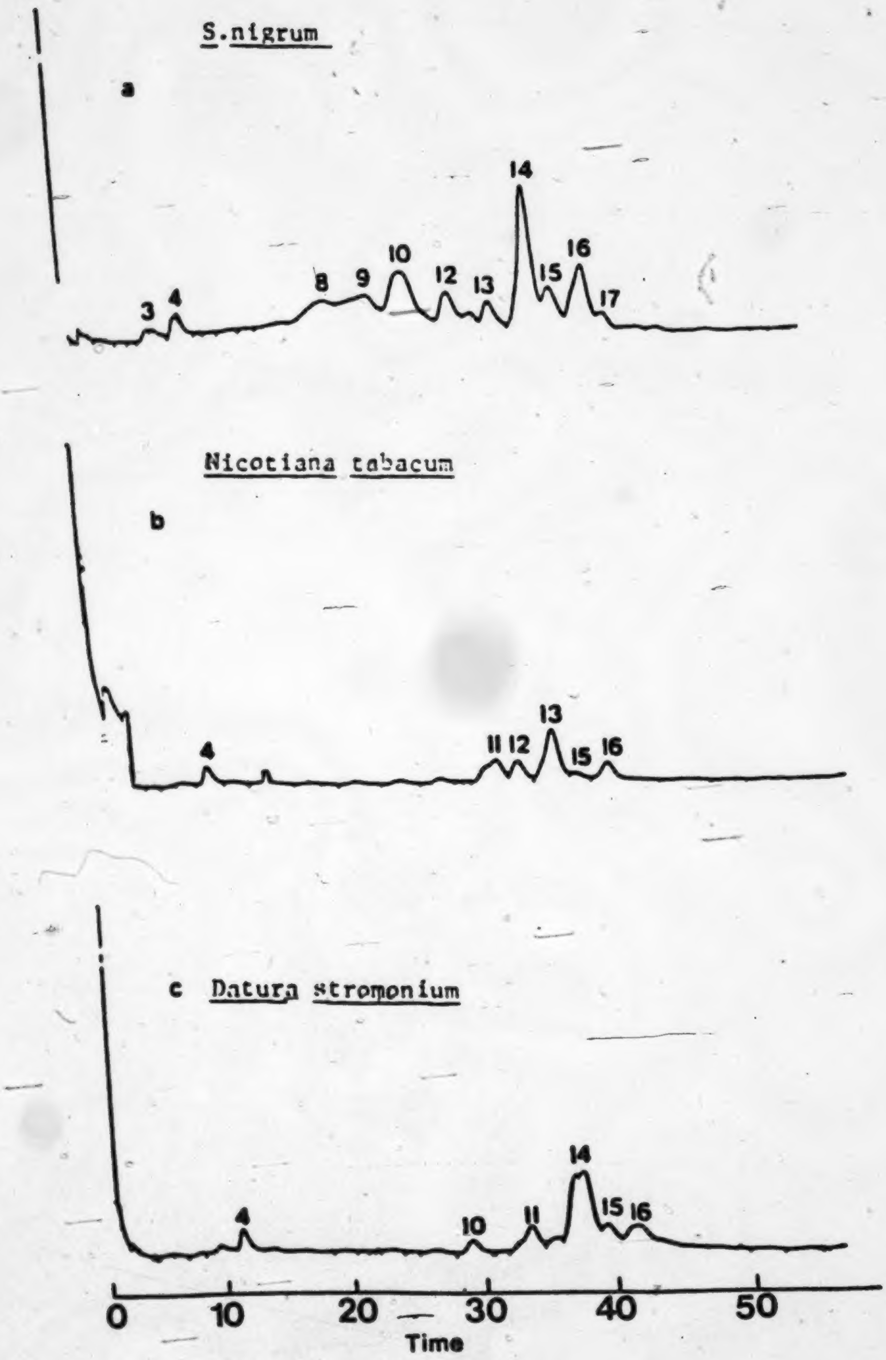


S.tuberosum

Lycopersicon esculentum

S.elaeagnifolium

**Fig. 4.5** : GLC profile of epicuticular waxes isolated from leaves of a) *S.nigrum* b) *N.tabacum* and *D.stromonium*. 1-8 : *p*-alcohols, aldehydes, ketones and *s*-alcohols; 9,11,12,14: alkanes; 15-18:esters.

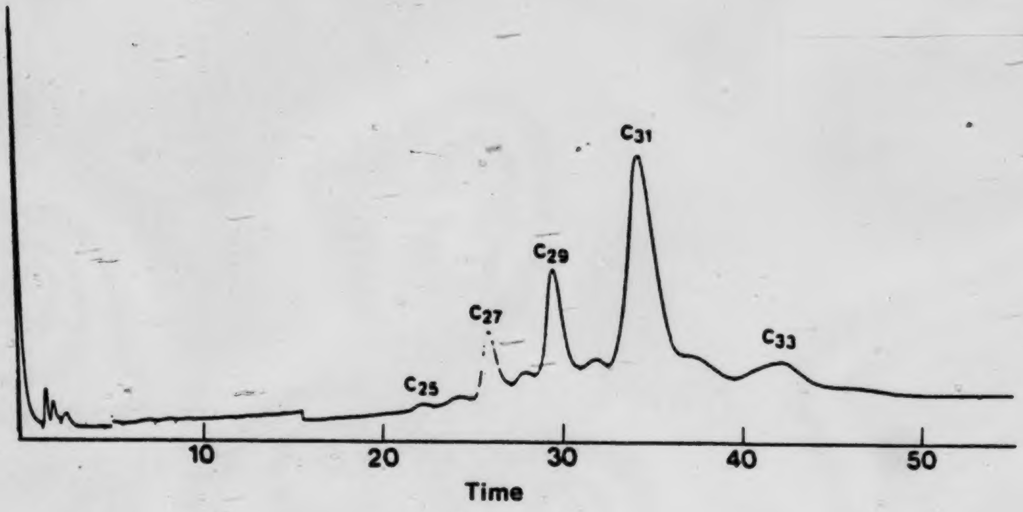


S.nigrum

Nicotiana tabacum

Datura stromonium

**Fig. 4.6** : GLC profile of the *n*-alkane fraction from leaf surface waxes of *S.tuberosum.*


$C_{25}$
$C_{27}$
$C_{29}$
$C_{31}$
$C_{33}$
10
20
30
40
50
Time

## REFERENCES

Baker,EA (1974) The influence of environment on leaf surface wax development in *Brassica oleracea* var. *gemmifera*. New Phytol.73: 955-966.

Baker,EA (1982) Chemistry and morphology of plant epicuticular waxes. In: The Plant Cuticle. DF Cutler, KL Alvin and CE Price (eds.).pp: 139-165. Academic Press,New York.

Baker,EA and Procopiou,J (1980) Effect of soil moisture status on leaf surface wax yield of some drought resistant species. J.Hort.Science 5:85-87.

de Bary,A (1871) Ueber die Wachsiiberzüge der Epidermis. Botanisches Zeitung. 29: 145-154.

Bianchi,A and Marchesi,G (1960) The surface of the leaf in normal and glossy maize seedlings. Zeit. für Verebungslehre 91: 214-219.

Bukovac,MJ, Flore,JA and Baker,EA (1979) Peach leaf surfaces : changes in wettability, retention, cuticular permeability and epicuticular wax chemistry during expansion with special reference to spray application. J.Amer.Soc.Hort.Sci.104: 611-617.

Carruthers, W and RAW Johnstone (1959) Composition of paraffin wax fraction from tobacco leaf and tobacco smoke. Nature.184: 1131-1132.

Crisp,CE (1966) The plant epidermis. Western Weed Control Conference Progress Report: 137-138.

Denna,DW (1970) Transpiration and the waxy bloom in *Brassica oleracea* L. Aust.J.Biol.Sci.23: 27-31.

Flore,JA and Bukovac,MJ (1978) Pesticide effects on the plant cuticle : III. EPTC effects on the qualitative composition of *Brassica oleracea* L. leaf cuticle. J. Amer. Soc. Hort. Sci. 103: 297-301.

Grncarevic,M and Radler,F 1967. The effects of wax components on cuticular transpiration-model experiments. Planta(Berl.).75:23-27.

Hadley,NF (1981) Cuticular lipids of terrestrial plants and arthropods : a comparision of their structure, composition and water proofing function. Biol.Rev.36: 23-47.

Hall,DM and Davidson,LA (1962) Secretion from pores of surface wax of plant leaves. Nature. 1194-1196.

Hallam, ND (1970) Growth and regeneration of waxes on the leaves of Eucalyptus. Planta(Berl.) 93:257-268.

Herbins,GA and Robins,PA (1969) Patterns of variation and development in leaf wax alkanes. Phytochem. 8: 1985-1998.

Holloway,PJ (1970) Surface factors affecting the wetting of leaves. Pesti. Science. 1:156-163.

Holloway,PJ, Brown,GA, Baker,EA and Macey,MJK (1977) Chemical composition and ultrastructure of the epicuticular wax in three lines of *Brassica napus* (l.). Chem Phys. Lipids 19: 114-127.

Jarvis,LR and Wardrop,AB (1974) The development of the cuticle in *Phormium tenax*. Planta 119: 101-112.

Jeffree,CE, Baker,EA and Holloway,PJ (1975) Ultrastructure and recrystallization of plant epicuticular waxes. New Phytol.75: 539-549.

Jeffree,CE Baker,EA and Holloway,PJ (1976) Origins of the fine structure of plant epicuticular waxes. in Microbiology of aerial plant surfaces. pp: 119-158. C.H.Dickinson and T.F.Price (eds.). Academic Press, London.

Jeffree,CE, Johnson,RPC and Jarvis,PC (1971) Epicuticular wax in the stomatal antechamber of Sitka spruce and its effect on the diffusion of water vapour and carbon dioxide. Planta(Berl.) 98:1-10.

Juniper,BE (1960) Growth, development and effect of environment on the ultrastructure of plant surfaces. J.Linn.Soc.(Botany).56:413-419.

Knowles,LO and Flore,JA (1983) Quantitative and qualitative characterization of carrot root periderm during development. J.Amer.Soc. Host.Sci. 108:923-928.

Kolattukudy,PE (1966) Biosynthesis of wax in *Brassica oleracea*. Relation of fatty acids to wax. Biochemistry 5(7): 2265-2268.

Kolattukudy, PE (1970) Compositon of the surface lipids of pea leaves (*Pisum sativum*). Lipids 5(4) :398-402.

Kolattukudy, PE (1975) Biochemistry of cutin, suberin and waxes, the lipid bariers on plants. In : Recent advances in the chemistry and biochemistry of plant lipids. T Gaillard and EI Mercer (eds.). pp: 203-246.

Academic Press, New York.

Kollatukudy, PE and Walton,TJ (1972) The biochemistry of plant epicuticular lipids. in Progress in the chemistry of fats and lipids. XIII(3): 119-175.

Leigh,JH and Matthews,JW (1963) An electron microscope study of the wax bloom on leaves of certain love grasses (*Eragostis curvula* (Shrad.)Nees). Aust.J.Botany 11: 62-66.

Lundqvist,U, von Wettstein-Knowles,P and von Wettestein,D (1968) Induction of eceriferum mutants in barley by ionizing radiations and chemical mutagens. Hereditas. 48 : 342-362.

Lundqvist,U and von Wettestein,D (1968) Induction of eceriferum mutants in barley by ionizing radiations and chemical mutagens. Hereditas. 59: 473-504.

Martin,JT and Juniper,BE (1970) The cuticle of plants. Edward Arnold. London.

Muellar,LE, Carr,PH and Loomis,WE (1954) The submicroscopic structure of plant surfaces. Am.J.Bot.41: 593-600.

Pady,SM (1971) Spore release in some foliar saprophytes and parasitic fungi. in Ecology of leaf surface microorganisms. T.F.Preece and C.H.Dickinson (eds.). pp.111-115. Academic Press,London.

Stoianova-Ivanova,B and Mladeva,K (1971) The composition and structure of ketones from rose bud amd rose flower waxes. Phytochem. 10 : 1391-1393.

Tukey, HB (1971) Leaching of substances from plants. In : Ecology of leaf surface microorganisms. TF Preece and CH Dickinson (eds.) PP. 67-80. Academic Press, London.

Tulloch, AP (1976) Chemistry of waxes of higher plants. In : Chemistry and Biochemistry of Natural Waxes. PE Kolattukudy, (eds.) pp. 235-277. Elsevier, Amsterdam.

Tulloch, AP (1983) Epicuticular waxes from *Agropyron dasystachyum, Agropyron riparium* and *Agropyron elongatum*. Phytochem. 22: 1605-1613.

Wettstein, Knowles,P von (1974) Ultrastructure and origin of epicuticular wax tubes. J.Ultra.Res.46: 483-498.

Wiesner,J (1871) Beobachtung über die Wachsüberzüge du Epidermis. Botanisch. Zeitung. 29:769-774.

Wollrab,V (1969) Secondary alcohols and paraffins in the plant waxes of the family Rosaceae. Phytochem. 8:623-627.

## V. Influence of leaf surface waxes on the feeding behavior of Colorado potato beetle, *Leptinotarsa decemlineata* (Say)

### INTRODUCTION

While extensive studies have been carried out on insect-host plant relationships, there have been few attempts to study in detail the importance of leaf surface waxes to host selection behavior of insects. It has been shown in locusts and grasshoppers that leaf surface waxes influence host plant selection and that sensilla on the maxillary palpi perceive the stimulus. Bernays *et al* (1976) provided evidence that leaf surface waxes of *Poa annua* promote biting activity in nymphs of *Locusta gregaria*. Adults of the same species could also differentiate between waxes of *Poa annua* and *Bellis perennis* (Blaney and Chapman,1970). Certain plants are rejected by the grasshoppers, *Chorthippus parallelus* and *Chortoicetes terminifera*, following contact with the leaf surface by terminal sensilla on the maxillary palpi (Bernays and Chapman,1970;1973). In *Locusta migratoria*, resistance to seedling sorghum is partly due to presence of an antifeedant *p*-hydroxybenzaldehyde in leaf surface waxes (Woodhead,1982).

The ability to perceive and respond to leaf surface waxes has also been recorded in *Acyrthosiphon pisum* (Klingauf *et al*,1971); *Manduca sexta* (De Boer,1985); *Choristoneura fumiferana* (Maloney,1985) and *Psila rosae* (Städler and Büser,1984). Such waxes also influence the searching behavior of a predaceous beetle, *Adalia bipunctata*, where movement is restricted to leaf edges or protruding veins (Shah,1982).

There is conclusive evidence suggesting that antennae function over distance in limited host plant selection by the adult Colorado potato beetles (de Wilde,1958;1976; Visser,1979). It is believed that palpation observed in adult beetles is probably a mechanism by which adult beetles recognize host plants by identifying species-specific leaf surface chemicals. The main objective of this chapter was to test this hypothesis and to study the role of palpi in host plant selection over short distances.

## MATERIALS AND METHODS

Plants used in these experiments were grown in the greenhouse with day length maintained at a minimum of 16 hours using fluorescent fixtures or Sonline high intensity sodium lamps as supplemental lighting. *S.tuberosum* was grown from tubers while *L.esculentum, S.nigrum, S.elaeagnifolium, D.stromonium* and *N.tabacum* were all grown from seeds. To minimize effects due to interplant variability, leaves from at least five plants of each species were used in behavioral studies. All leaves were taken from healthy plants and only leaves from the second or third node below the tip of a stem were used.

Insects used in this experiment were obtained from a culture maintained in this laboratory at 16L:8D, $25^0C \pm 3^0C$ under a combination of fluorescent and incandescent light. Insects were reared on potato leaves.

Behavioral studies were conducted with freshly emerged (naive) beetles, ie, with no feeding experience and in their first day after emergence. Older beetles had feeding experience. Leaf discs were cut with a cork borer. Bioassays with leaf surface waxes and their fractions were done using millipore filter discs. These were immersed in extract, allowed to dry and then were exposed to the beetles for testing. Waxing of palpi was done by depositing a very fine layer of beeswax on the tip of each palp using a heating element. Care was taken to apply as thin a layer as possible since the mass of large amounts restricted movement. Such beetles were allowed to adjust to this condition for two hours in petri dishes lined with moist filter paper. After completion of the experiment, each beetle was observed under a binocular microscope to confirm the presence of wax on the palpal tip.

### BEHAVIOR PROTOCOLS

Once in physical contact with the leaf, the adult beetle follows a stereotyped and ordered pattern of sampling, feeding, grooming and rest. The initial stages of sampling and feeding are discussed in this chapter: when a beetle arrives on the leaf surface, its maxillary palpi 'palpate' (repetitive contacts by the maxillary palpi on the leaf surface) and its antennae wave. During this period, the labial palpi are in continuous contact with the leaf and the mandibles squeeze the leaf often before finally taking a small bite (Harrison, 1985). A small bite differs from the several squeezes the mandibles make in that a small portion of the leaf is ingested into the oral cavity. This is followed by a feeding bout which can last several minutes and eventually a period of rest. In all behavioral studies discussed in this chapter, the time (in seconds) taken by the beetle to take the first bite following its initial contact is the unit of measure unless otherwise indicated. Statistical analysis was done using the Scheffe's F-test.

## RESULTS

In preliminary studies of older beetles feeding on potato leaves in petri dishes, time taken for the first bite in females (Mean=8.02 sec, SE=0.545, n=10) and males (Mean=12.42, SE=2.398, n=10) did not differ significantly (F=3.48; df=1,18; P>0.001). Under similar conditions, no sigificant differences were observed between males and females of naive beetles (Females: Mean=20.58 sec, SE=5.02, n=10; Males: Mean=18.96 sec, SE=4.39, n=10; F=0.06, df=1,18, P>0.01). Therefore, because supplies of new adults were limited, both sexes were used in behavior tests.

Beetles with their palpi waxed took significantly longer to take in the first bite as compared to naive and older beetles (F=18.04, df=2,57, P<0.001) (Table 5.1). There was thus an increase in sampling behavior in such beetles. Palpation was more frequent and feeding was observed at numerous sites compared to those of naive and older beetles. There were no significant differences between naive (Mean=19.77,SE=3.25) and older beetles (Mean=10.2,SE=1.29) (F=2.152, df=2,57, P>0.05) (Table 5.1).

To study the order of preference of beetles between three host plant species and three non host plant species, all belonging to the Solanaceae, the time taken for first bite and for first meal were recorded. Termination of first meal was defined as a cessation of feeding for more than 30 seconds and with the beetle either grooming, resting or off the leaf disc.

Table 5.2 lists the plant species ranked in order of least to greatest amount of time taken for first bite. On non preferred host plants like *N.tabacum* and *L.esculentum*, the stereotyped behavioral pattern was not observed with an increase in sampling behavior. Instead, the mandibles were used to squeeze the leaf at numerous sites before a bite was taken. On preferred plants, the beetles went through the entire sequence of sampling and usually took the first bite at the site where the mandibles first squeezed the leaf tissue. Time taken by beetles for the first bite on *N.tabacum* was significantly higher than on any of the other plant species, but the remaining times did not differ significantly.

Data on time taken for first meal presented an interesting pattern (Table 5.3). On average, longer feeding was observed on the three host plants, viz.,*S.tuberosum*, *S.elaeagnifolium* and *L.esculentum*. Differences were significant between *S.tuberosum* and the other species as well as between *S.elaeagnifolium* and *S.nigrum*. On non host plants, there were longer gustatory sampling periods,ie, several bites being taken by a beetle before it eventually settled on a feeding bout.

Crude leaf surface waxes, extracted as described in Chapter IV, were exposed to beetles on millipore filter discs at 5 times the concentration present in individual leaves and along with 0.025M sucrose. The time for the first bite was again observed (Table 5.4). All beetles were observed individually for a maximum period of 25 minutes. Time taken for first bite was significantly longer on the control disc containing only 0.025M sucrose than for the test disks containing crude waxes from any of the plant species tested (F=41.57, df=6,133, P<0.001). Table 5.4 lists the species ranked from least to greatest amount of time taken for first bite on crude waxed discs. Interestingly, the order of preference for the six species was very similar to that obtained when beetles were offered leaf discs from the plants. Beetles exposed to treated millipore filter discs were observed to make numerous bites with their mandibles. Movements of antennae were more rapid than on plants as was palpation by the maxillary palpi.

Fractions from potato leaf surface waxes were exposed to beetles to identify components which are stimulatory (Table 5.5). The hexane fraction appeared inactive and hence was not considered any further. Table 5.5 lists the times taken for the first bite on different fractions of potato. On average, durations were much higher than when potato crude waxes were offered (Table 5.4). The benzene and chloroform extract differed significantly (F=5.63, df=2,57, P<0.01).

## DISCUSSION

The primary objective of this behavioral study was to investigate mechanisms of host plant discrimination involving short distances by *L.decemlineata* adults. Using behavioral analyses, the role of maxillary and labial palpi were investigated in this regard and the beetles response to leaf surface waxes was investigated in particular.

In some cases, hunger may be a biting stimulant in itself leading to erroneous results. Blaney and Chapman (1970) observed, that after prolonged starvation, biting becomes indiscriminate. The results obtained show that a) naive beetles do not interfere with preference tests; b) use of both males and females do not bias results; and that discs with c) leaf surface waxes enhanced feeding compared to control discs.

Behavioral studies indicate that the beetles are capable of graded, sensory based discrimination of plants over a range of plant species normally encountered in nature. Based on the data on time taken for first bite and first meal, an interesting pattern of relationship exists between time spent in sampling behavior and duration of first meal. On non-preferred plants, an increase in sampling behavior suggests the action of a complex of stimuli involved in plant discrimination. It has been shown that adult *L.decemlineata* are capable of fine olfactory discrimination of plants. Visser and Avé (1978) have shown that long range orientation to a host is dependent on particular ratios of green leaf volatiles which are coded by olfactory receptors on the antennae. In general, input from mouthpart sensilla in phytophagous insects typically converge with antennal input from the brain after only one synapse in the sub-esophageal ganglion.

Short durations of first meal on non preferred plants also suggests the involvement of general leaf chemicals. Leaf sap exudates released by the first bite or even by the squeezing action of the mandibles allows plant chemicals such as alkaloids to be encountered by sensilla on the galea and epipharynx and also releases additional volatiles. *S.nigrum* is characteristic in containing tropane alkaloids. Kogan (1976) suggested that *L.decemlineata* adults may be physiologically adapted to respond to steroidal alkaloids like tomatine but not to tropane alkaloids, thereby allowing expansion of the species host range to include tomato but not species like *Datura stromonium* or *S.nigrum.*

Behavioral studies with crude wax extracts and with different fractions from potato leaf surface waxes indicate that waxes enhance feeding. Significant differences for first bite did not occur between crude wax extracts from the different solanaceous species investigated despite their different chemical profiles (Chapter IV). The chemical constituents of wax are similar in all these species although the individual amounts of their components vary. An almost similar ranking of plants when offered either leaf discs or crude waxes suggests that once on the leaf/substrate, sensory information necessary for taking the first bite is obtained from the surface waxes. An analysis of surface waxes (Chapter IV) shows that most of the components are volatile and since the maxillary palpi are primarily olfactory in terms of their sensory modality, it seems reasonable to assume that palpation causes turbulence within the head space area. Volatiles from leaf surface waxes are then perceived by the olfactory sensilla on the palpi thereby causing a behavioral response seen as the first bite.

Behavioral studies with the different fractions of potato leaf surface waxes lend further support to this idea. The chloroform fraction, essentially consisting of esters and alcohols, stimulated the beetles more than the benzene and methanol fractions. Blaney and Chapman (1970) mentioned that 'biting is not a non-specific reaction as is commonly believed'. These results lend support to the idea that waxes act as 'biting stimulants' and thereby induce feeding though they probably do not maintain feeding as sucrose, for example, does.

Behavioral response to changes in wax concentration by 5 fold could be interpreted either as a) increased preference; b) decreased preference or c) no effect on preference. Logically, a) must indicate increasing concentration of the stimulant, while b) might indicate increasing concentration of a feeding deterrent. However, this is speculation. The interactions are much more complicated than indicated by this simplistic analogy. Leaf surface waxes are present in very minute amounts and their perception and especially their discrimination by adult beetles inspite of mixing of stimuli by wind turbulence, implies a high degree of sensory sophistication. Certain compounds may have narrow optimal concentration ranges whereby a behavioral protocol is triggered. In any case, the above three categories will serve as a starting point for further research.

**Table 5.1** : Average time taken by naive beetles, older beetles and naive beetles with their papi waxed to take the first bite when offered potato leaves. Means represented by the same letters are not significantly different at $P<0.01$ ($n=20$).

| | Time (sec)<br>Mean ± S.E. | |
|---|---|---|
| Naive | 19.77±3.25 | (A) |
| Older | 10.22±1.29 | (A) |
| Palpi waxed | 37.47±5.19 | (B) |

**Table 5.2** : Average time taken by beetles to take the first bite on different solanaceous species. Means represented by the same letters are not significantly different at $P<0.01$ $(n=20)$

| Plant species | Time (sec)<br>Mean ±S.E. | |
|---|---|---|
| *S.tuberosum* | 6.97± 0.64 | (A) |
| *S.elaeagnifolium* | 7.70± 0.83 | (A) |
| *D.stromonium* | 8.30± 1.01 | (A) |
| *S.nigrum* | 8.45± 1.15 | (A) |
| *L.esculentum* | 10.12 ±1.03 | (A) |
| *N.tabacum* | 17.22±0.99 | (B) |

**Table 5.3** : Average time taken for the first meal by adult *L.decemlineata* beetles when offered leaf discs of different solanaceous plants. Means represented by the same letters are not significantly different at $P<0.01$ ($n=20$).

| Plant species | Time (sec) Mean ±S.E. |
|---|---|
| *S.tuberosum* | 251.35±52.46 (A) |
| *S.elaeagnifolium* | 132.30±19.78 (B) |
| *L.esculentum* | 48.25±10.85 (B) |
| *D.stromonium* | 35.55± 3.70 (B) |
| *N.tabacum* | 32.15± 2.96 (B) |
| *S.nigrum* | 14.90± 1.93 (C) |

**Table 5.4** : Average time taken by the beetles to take the first bite when offered crude leaf surface waxes on millipore discs from different solanaceous species. Means represented by the same letters are not significantly different at $P<0.01$ (n=20).

| Plant species | Time (sec) Mean ±S.E. |
|---|---|
| *S.tuberosum* | 398.85±41.71 (A) |
| *S.elaeagnifolium* | 434.55±47.09 (B) |
| *D.stromonium* | 593.90±47.89 (B) |
| *N.tabacum* | 621.45±54.94 (B) |
| *S.nigrum* | 664.15±47.41 (B) |
| *L.esculentum* | 706.50±95.27 (C) |
| Control | 1465.25±95.27 (D) |

**Table 5.5** : Average time taken by the beetles for the first bite when offered different fractions of potato leaf surface waxes on millipore discs. Means represented by the same letters are not significantly different at $P<0.01$ ($n=20$)

| Wax Fractions | Time (sec)<br>Mean ±S.E. |
|---|---|
| Chloroform | 411.90±51.91 (A) |
| Methanol | 545.90±74.71 (A) |
| Benzene | 655.75±56.18 (B) |
| Control | 1465.25±95.27(c) |
| Hexane | >1500 |

## REFERENCES

Bernays,EA, Blaney,WM, Chapman,RF and Cook,AG (1976) The ability of *Locusta migratoria* to perceive plant surface waxes. Symp. Biol Hung. 16: 35-40.

Bernays,EA and Chapman RF (1970) Food selelction by *Chorthippus parallelus* (Zetterstedt) (Orthoptera: Acrididae) in the field. J.Anim.Ecol. 39:383-394.

Bernays,EA and Chapman,RF (1973) Food in survival and development of *Chortoicetes termifera* in drought conditions. Aust.J.Zool. 21:575-592.

Blaney, WM, and Chapman,RF (1970) The functions of the maxillary palps of Acrididae (Orthoptera). Ent.exp.appl. 13: 363-376.

De Boer,G (1985) The role of phytochemicals, chemosensory organs, and feeding experience in food plant selection by the larvae of the tobacco hornworm, *Manduca sexta* (Lepidoptera:Sphingidae). Ph.D Dissertation; University of Maryland, Baltimore, USA.

Harrison,GD (1985) Host plant discrimination and the evolution of feeding preference in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). M.Sc. Thesis, University of Alberta,Edmonton, Canada

Klingauf,von F, Nocker-Wenzel,K and Klein,W (1971) Einfluss einigers Wachskomponenten von *Vicia faba* auf das Wirtswahlverhalten von *Acyrthosiphon pisum* (Harris)(Homoptera:Aphididae). Zeit. Pflanzenkrank. Pflanzensc. 78:641-648.

Maloney, PJ (1985) Influence of epicuticular waxes from white spruce and balsam fir on feeding behavior of the Eastern spruce budworm~~. M.Sc Thesis.~~ Concordia University, Montreal.

Kogan,M (1976) The role of chemical factors in insect/plant relationships. Proc.XV Int. Cong Entomol.pp: 211-227.

Städler,E and Büser,H -R (1984) Defense chemicals in leaf surface wax synergistically stimulate oviposiiton by a phytophagous insect. Experientia 40: 1157-1159.

Shah,MA (1982) The influence of plant surfaces on the searching behavior of coccinelid larvae. Ent.exp.appl. 31:377-380.

Visser,JH (1979) Electroantennogram responses of the Colorado beetle, *Leptinotarsa decemlineata*, to plant volatiles. Ent.exp.appl. 25 : 86-97.

Visser,JH and Ave,DA (1978) General green leaf volatiles in the orientation of the Colorado betle, *Leptinotarsa decemlineata*. Ent.exp.appl. 24:738-749.

de Wilde,J (1958) Host plant selection in the Colorado beetle larva,(*Leptinotarsa decemlineata* Say). Ent.exp.appl. 1: 14-22.

de Wilde J (1976) The olfactory component in host-plant selection in the adult Colorado beetle, (*Leptinotarsa decemlineata* Say). Symp.Biol.Hung. 16(2): 291-300.

Woodhead,S (1982) *p*-hydroxybenzaldehyde in the surface wax of sorghum : its importance in sedling resistance to acridids. Ent. exp.appl. 31: 296-302.

# VI. CONCLUDING DISCUSSION

Behavioral elements which constitute the complex feeding process of an insect depend on the insect receiving various sensory stimuli from the plant. Specifically, before nutritional and other factors become operative, the insect must first sense and discriminate (Dethier,1970).

Current hypotheses on mechanisms underlying host choice by insects include dynamic concepts involving the integration of patterned sensory input and the perception of total plant '*Gestalts*' (Dethier,1970,1982). Most insects probably choose plants on the basis of complex patterns of sensory information gathered across several modalities incuding gustation, olfaction, vision and mechanoreception (Dethier, 1982; Miller and Stickler,1984). This ability endows them with the potential for perceiving and responding to subtle variations in plant quality.

Orientation of an insect towards its host plant is the first step in host-plant selection. In *L.decemlineata* adults, the antennae detect general green leaf volatiles from potato foliage which orient the beetle towards its host. Alteration of the blend with unnatural concentrations can destroy the orientation (Visser and Thiery,1985).

The preceeding chapters clearly demonstrate the importance of maxillary and labial palpi in host-plant selection by adult beetles. Presumably these sensory organs function over distances from a few mm to direct contact. Waxing the palpi results in blocking sensory information resulting in longer times to take the first bite (ie. extended sampling behavior). Since the maxillary palpi are primarily olfactory in function (Chapter II), it is suggested that they detect specific volatiles in the head space of host-plants, thereby contributing to initiate the first bite. Palpation observed in adult beetles before taking a bite probably provides ventilation (analogous to sniffing). It is not certain if sensilla are stimulated during the brief contact or if volatile compounds constantly diffuse into the sensillar pores producing a continual stimulation. It has been observed that palpation reduces the rate of sensory adaptation in the migratory locust, *Locusta migratoria* (Blaney and Duckett,1975) and in the silkworm, *Bombyx mori*, that olfactory inputs from basiconic sensilla on the maxillary palpi either activates or inhibits mandibular biting activity by modifying a centrally generated motor program (Hirao *et al*,1976).

The labial palpi and galeal sensilla may help to maintain feeding. The labial palpi are in continuous contact with the leaf surface during feeding while the galea come into intermittent contact with the leaf sap once the beetle has taken a bite.

Leaf surface waxes of potato have been shown to be stimulatory to this insect and it is suggested that volatiles, essentially esters, primary and secondary alcohols act as 'biting stimulants'. Leaf surface wax components probably also act as feeding co-factors since the labial palpi are in continuous contact with the leaf during feeding. The present study suggests that *L.decemlineata* adults are capable of perceiving its own blend (mixtures) of compounds which may typify each plant as evidenced by different feeding rates on six solanaceous species, although the blends are similar enough that they all act as biting stimulants. The ratios of various components within the blend is responsible for the different feeding rates.

It would be interesting to compare both qualitative and quantitative analyses of leaf surface waxes using combined GC and electrophysiological techniques. It might then be possible to identify components which form the 'chemical image' of the host. Such an approach coupled with the behavioral studies reported here would lead to a better understanding of host selection and its possible manipulation.

## REFERENCES

Blaney, WM and Duckett,AM (1975) The significance of palpation by the maxillary palps of *Locusta migratoria* (L.) : an electrophysiological and behavioral study. J.exp.Biol. 63:701-712.

Dethier, VG (1970) Chemical interactions between plants and insects. In : Chemical Ecology. E. Sondheimer and J.B.Simeone (eds.) pp: 83-102. Academic Press, NY.

Dethier, VG (1982) Mechanism of host-plant recognition. Ent.exp.appl. 31: 49-56.

Harrison,GD (1985) Host plant discrimination and the evolution of feeding preference in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). M.Sc Thesis, University of Alberta, Edmonton. 84pp.

Hirao,T, Yamaoka,K and Arai,N (1976) Studies on mechanism of feeding in the silkworm, *Bombyx mori* L.II. Control of manibular biting movement by olfactory information through maxillary sensilla basiconica. Bull. Sericul.Exp. Sta. 122: 417-418.

Miller,JR and Strickler,KL (1984) Finding and accepting host plants. In : Chemical Ecology of insects. W.J.Bell and R.T.Cardé. (eds). pp: 127-157. Sinauer Associates, Sunderland, Mass.

Visser,JH and Thiery,D (1985) Behavioral responses of the Colorado potato beetle to stimulation by wind and plant odors. In : Proc.Symp.Colorado potato beetle, XVIIth. Int.Congr.Entomol. DN Ferro and Voss,RH (eds.) :117-125.